

LEPTOSPIROSIS IN THE BRITISH ISLES.

LEPTOSPIROSIS IN THE BRITISH ISLES

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Containing

A General History of the Epidemiology and Bacteriology of

Leptospirosis Ictero-haemorrhagiae

and

Leptospirosis Canicola,

with Special Reference to the Occurrence

of the Infections in the

British Isles

and

to the Writer's Personal Experience of them

during Fifteen Years.

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LEPTOSPIROSIS IN THE BRITISH ISLES.

AN EPIDEMIOLOGICAL AND BACTERIOLOGICAL STUDY.

PART I. LEPTOSPIROSIS ICTERO-HAEMORRHAGIAE (WEIL'S DISEASE).

HISTORICAL.

The separation of leptospiral jaundice from other infectious diseases of the liver occurred in two stages - the first, clinical and nearly thirty years later the second, bacteriological.

In 1886, Professor Adolf Weil, of Heidelberg, described four cases of a form of infectious jaundice. Two had occurred in 1870 and two in 1882, and they were all so similar that he considered them to be of the same disease. The occupations of the four men were chemist, soldier, shop-keeper and waiter. In each of the patients there was a febrile illness with severe nervous symptoms, enlargement of the spleen and liver, jaundice and signs of renal disease and, after a severe but relatively short course, recovery was rapid. Recurrence of temperature occurred for five or six days in three patients, following a remission of fever of one to seven days. In differential diagnosis, Weil carefully considered the recognised diseases of (i) catarrhal jaundice, (ii) primary nephritis, (iii) a combination of catarrhal jaundice and primary nephritis, (iv) yellow atrophy of the liver, (v) pyaemic or septicaemic fever, (vi) recurrent fever (vii) bilious typhoid fever. He suggested that the cases represented a new entity, although he could demonstrate neither its anatomical basis nor the infective agent.

During the next thirty years the name of Weil's disease was used in all parts of the world, but mostly in Germany, to describe a febrile illness with jaundice in epidemic or endemic form, but there was doubt as to its applicability in individual cases or even its justification in general.

As early as 1889, E.H. Young reported in "The Lancet", as an example of Weil's disease, a man who was taken ill two days after returning from Yeomanry training in S.W. England. Young gave an excellent description of the disease from day to day, describing drowsiness, suppression of urine, tarry stools, jaundice and injection of the eyes. Five days of acute illness was followed by recovery. An origin at the Yeomanry camp was suspected.

In 1892, H. Jaeger reported nine cases among soldiers in the garrison at Ulm. He attributed their illness to bathing in a river near the town. Three of the patients died and he cultured from the organs of two of them *Bacillus proteus fluorescens* which he suggested might be the causal organism of the disease.

William Hunter (1908) wrote that most French and English writers had declined to recognise Weil's disease as separable from icterus gravis or infectious jaundice and he agreed with that opinion. However, mainly from German reports of the disease he gave a detailed account of the characters alleged to belong to it. These included the clinical features given above from Weil's own report, with emphasis on the severity

of muscular pains and of nephritis; enlargement of the spleen was said to be usual. Etiological features were that the incidence was mostly in boys and men of 15 to 30 years of age; by some estimates 90 per cent of the patients were male. No absolute class distinction could be made but the most frequent patients were working men whose occupation or habits exposed them to insanitary surroundings. Hunter quoted thirteen cases (of which nine were in men working in a slaughter house in Dresden) and the nine instances quoted above (Jaeger, 1892), traced to bathing by soldiers in a river near Ulm. Cockayne (1912) in a good review, clearly separated epidemic catarrhal jaundice from infectious jaundice or Weil's disease. He believed that the former is much the commoner and much milder, is almost world-wide in distribution and is air-borne; and that Weil's disease is probably due to contaminated water or food, but may be due to a biting insect and is met with chiefly round the Mediterranean Sea and does not extend to the British Isles. His opinion about the special liability of the Mediterranean area was due to such reports as that of Sandwith (1904) who described an endemic and epidemic disease resembling Weil's disease in Smyrna. Sandwith did not consider Weil's disease was a separable entity. In 1915, Boggs was equally sceptical when he stated: "Weil's disease is a type of infectious jaundice in which fever, enlargement of the spleen and liver, nephritis and muscular pain accompany the icterus. But there are so many similar sporadic and epidemic cases in which one or more of these features may be wanting that it seems hardly justifiable to separate this group sharply from

all the others". This scepticism was unjustified in the light of the discovery of the causative organism of this form of jaundice in 1915 and the failure in clinical acumen seems to be rather conspicuous when the description of the illness and the etiological features enumerated by Hunter are considered. It would appear that the success of bacteriology had made clinicians doubtful of their own judgment.

Japanese clinicians and pathologists had been convinced of the existence of Weil's disease as a separate form of jaundice before some of them discovered the infective organism. R. Inada and others (1916) wrote that the illness was, at that time, well known in various parts of Japan as an endemic and epidemic occurrence characterised by conjunctival congestion, muscular pain, fever, jaundice, haemorrhagic diathesis, albuminuria and a fairly high mortality rate. They stated that splenic enlargement was rare (10 per cent in their clinic). In view of later experience in Europe it would seem that before this time European clinicians had exaggerated the increase of the spleen [and, if this is so, the justification of distinguishing Weil's disease on clinical grounds is made clearer].

It was in November, 1914, at the Imperial University in Kyushu, that R. Inada and his colleagues (1916) first saw a spirochaete in the liver tissues of a guinea-pig which had been injected with blood from a patient with Weil's disease. By repetition of this result from thirteen other patients with the disease and by the absence of such findings in guinea-pigs injected with blood from other diseases, Inada and his colleagues



concluded in January, 1915, that they had found the causal organism and they named it *Spirochaeta ictero-haemorrhagiae*. They made excellent drawings of dark-ground preparations of the organism and made good photomicrographs of stained specimens. They grew the organism in successive cultures in Noguchi's medium, which they modified by the use of guinea-pig's kidney in place of rabbit's kidney and found that cultures grew better at 22-25°C. than at 15°C or 37°C. They demonstrated antibodies against their spirochaetes in the blood of five out of six patients. They showed that living spirochaetes could be found in the patients' urine for as long as 25 days after the illness began and that the abraded or intact skin allows entrance to the organism in guinea-pigs. These results were published first in 1915 in Japanese periodicals and in 1916 in the *Journal of Experimental Medicine*.

Ido and others (1916), working in the same team as Inada, reported that they found virulent *Spirochaeta ictero-haemorrhagiae* in the kidneys of 39.5 per cent of 86 house and ditch rats and they believed that they had accumulated sufficient knowledge of the source and modes of infection to apply prophylaxis. By disinfecting ground and removing stagnant water in certain places in coal mines, they considered that they had twice prevented epidemics of the disease. They experimented with active and passive immunisation in man and animals but did not achieve convincing success in preventive inoculation, which was their main object.

German workers published an independent, but not prior, discovery that Weil's disease is caused by a spirochaete which was named by Hübener and Reiter (1915, 1916) *Spirochaeta nodosa*, and by Uhlenhuth and Fromme (1916 a & b) *Spirochaeta icterogenes*. These names have not been used currently in English but they were accepted as synonymous with *Spirochaeta ictero-haemorrhagiae*. These German workers did not succeed so fully as the Japanese whose work has been described, but it is worth describing their results, which they announced before there is any likelihood that the reports in the Japanese periodicals reached them.

Hübener and Reiter reported that they had transmitted infection with the peripheral blood of at least seven men suffering from Weil's disease to guinea-pigs by intraperitoneal injection. The successes were mostly when blood was taken in the first 3 to 6 days of the illness and it produced in the animals the signs of Weil's disease seen in human beings, and caused death on the 5th to the 12th day with the morbid anatomy and histology of the human disease. They made twelve passages from guinea-pig to guinea-pig, injecting these animals per os and per anum. They successfully infected monkeys and rabbits. From the urine of a patient at the fifteenth day of illness they produced the disease in guinea-pigs. Later, by dark-ground illumination, they saw small, elongated structures with lashing motility and believed that these were the cause of the disease; they named them *Spirochaeta nodosa*. Uhlenhuth and Fromme confirmed Hübener and Reiter in the transfer of infection from man to guinea-pig and their special contribution at this stage was to be the first in Europe to demonstrate clearly in preparations



from the liver of guinea-pigs that there were active spirochaetes which could be seen in dark-ground preparations and in fixed films stained by Giemsa's or Levaditi's methods. They named the organism *Spirochaeta icterogenes*. [If these discoveries about the cause of Weil's disease had been made during the Second World War we should probably not have heard of the Japanese discoveries so rapidly as we did and, in that event, and by grace of holding the scales more evenly between two enemies (instead of between an ally and an enemy) we might have given relatively more credit to German independent work.]

In 1917 and 1918 (a & b), Noguchi made a series of careful morphological and cultural studies of the *Spirochaeta ictero-haemorrhagiae* of Inada and of strains from British cases of Weil's disease in Flanders and from wild rats in U.S.A. He found that from the three sources the organisms were the same in form and by immunological tests, and that they resembled no other organism already described except *Spirochaeta biflexa* which Wolbach and Binger (1914) had isolated in July 1913 by filtration from a fresh water pond in Massachusetts. On account of morphological differences from other spiral organisms, Noguchi named all these strains "*leptospira*" and distinguished them from those to which, on account of distinctive morphology, the generic names had already been given of "*spirochaeta*", "*saprosira*", "*cristispira*", "*spironema*" and "*treponema*". The generic name of "*leptospira*" has become more and more established since 1917. The original strain of *Leptospira biflexa* found by Wolbach and Binger did not survive its first subculture and although the name is used for some non-pathogenic saprophytic leptospiras, Lepto-

*spira ictero-haemorrhagiae* is the type species of the genus.

Morphology of Leptospira Ictero-Haemorrhagiae.

For a description of the causative organism of Weil's disease it would be difficult to improve on that given by Wolbach and Binger (1914) of *Spirochaeta biflexa*. These workers studied the organism alive and dead by dark-ground illumination and in films ~~stained~~<sup>fixed</sup> by osmic acid and stained by Giemsa's method. For the exact observation of morphology in as unimpaired a state of the organism as possible and for photography, they exposed drops of fluid containing the spirochaetes to the fumes of osmic acid and rapidly mixed them on a warm microscope slide with 3 per cent melted agar in water; a cover slip was put on and pressed down so as to form a very thin layer of jelly containing spirochaetes between the slide and cover. Their description of appearances by dark-ground illumination is as follows:-

"This spirochaete is characterised by the extreme closeness of the turns, its small size and curved or flexed extremities. The average length is from five to seven microns. The amplitude or width of the spirals measured from crest to crest is from 0.2 to 0.25 micron .... The two ends of the spirochaete are thinner than the body and taper to points. They are more or less sharply curved and sometimes have the form of a crook. When alive the spirochaete spins with extreme rapidity on its long axis in such a manner that the curved ends give the appearance of solid bulbs or flask-like bodies. In addition to this rapid rotation about the long axis there is fairly rapid translatory motion in either direction of the long axis. The living spirochaete is straight and presents the appearance of having a rigid long axis. Occasional slightly flexed forms which are motionless have been seen to straighten out and to begin to spin rapidly. In stained preparations the characteristics of the living organism are lost. The bodies become bent or flexed and the spirals less regular and less closely wound ..... The curved ends, giving unique appearances to the spirochaete in motion, are peculiar to this organism, and for this reason have been utilized in the construction of a name. The name *Spirochaeta biflexa* is proposed".

During recent years, leptospiras have been examined by the electron microscope. Morton & Anderson (1943) gained a resolving power of  $3\text{ m}\mu$  by this means and reported on appearances of *L. ictero-haemorrhagiae* and *L. canicola*. They observed that the length of *Leptospira ictero-haemorrhagiae* varied from  $4\mu$  to more than  $10\mu$  and could not determine the length of the longer cells since they extended over several fields. The width of the cell body was seen to vary from  $0.07$  to  $0.14\mu$ ; the spirals were about  $0.25\mu$  wide with a pitch of  $0.3$  to  $0.6\mu$ . These determinations agree reasonably with earlier observations by the visual microscope and the most interesting findings of Morton and Anderson were the negative ones that structures resembling flagella, such as were seen on some of the treponemes, were not seen on the leptospiras; also, unlike some treponemes, leptospiras did not show any granular internal structure or extruded granules. In some circumstances a sheath-like structure appeared round individual micro-organisms; this might be an artefact due to washing with distilled water or related to the cell wall of the bacteria.

#### The World-Wide Distribution of the Disease.

Weil's disease is widely distributed and, with some exceptions which will be noted below, has been identified in most regions of the world.

As was foretold by Inada, the spirochaetal cause of the disease was confirmed in British soldiers in Flanders (Stokes & Ryle, 1916; Dawson et al., 1917) and in French troops by Costa & Troisier (1916-17) and others. On the Italian front it was reported by Sisto (1917).

Since 1918 the disease has been recognised bacteriologically or serologically in most countries or regions of the world and reference is made here to some of the more notable, especially the earlier, publications on recognition of the infection. In Europe there are records for England (Manson Bahr, Wenyon & Brown, 1922; Alston & Brown, 1937), Scotland (Gulland & Buchanan, 1924; Davidson & Smith, 1939), Holland (Schüffner, 1934; Esseveld, 1937; Walch-Sorgdrager, 1939), Denmark (Zuelzer, 1936a; Petersen, 1944), France (Erber, 1934), Norway (Thjotta et al., 1939), Germany (Strasburger & Thill, 1929; Rimpau et al., 1938), Sweden (Malmgren, 1936), ~~Austria (Wirth, 1937)~~, Switzerland (Gsell, 1936), Dalmatia (Tarzaglia, 1939), Czechoslovakia (Bardos, 1936), Italy (Carlinfanti, 1942), Greece (Copanaris, 1932), Spain (Urtubey, 1929), Portugal (Jorge, 1932; De Azevedo, 1942) and Russia (Viskovsky, 1944). In Asia the infection has been identified in the Netherlands East Indies (Baerman, 1923), the Malay States (Fletcher, 1927), the Andaman Islands (Taylor & Goyle, 1931), French Indo-China (Bagiot & Delbove, 1934), India (Das Gupta and Chopra, R.N., 1937), Rangoon (Das Gupta, 1940) and China (T'ang, 1937). On the American continents the diagnosis has been established in the United States (Towler & Walker, 1927), Canada (Bates, 1926), Brazil (Piza & Gomes, 1930), Guadeloupe (Leger, 1932), Ecuador (Carbo-Neboa, 1924) and Argentina (Miyara et al., 1935). In Africa the infection was diagnosed on incomplete evidence by Melnotte and Farjot, (1927) in Morocco and reported from the Belgian Congo by Kadaner and Corti, (1934). In Australia, Queensland provided the first group of cases (Drew, 1934; Cotter, 1936), and other parts of the continent later (Sawers, 1938; Downes, 1942). **Patterson (1947) found 61 in Hawaii.**



Special attention has been given to the disease and a relatively large amount of it has been found as an endemic or epidemic condition in Japan, Holland and the Netherlands East Indies, the Andaman Islands and France. In Great Britain nearly 1,000 instances have been recorded since interest in the disease became intensified about the year 1933.

Investigation in the United States has been thorough since Wadsworth et al. (1922) reported the infection of a woman laboratory worker, who pricked her finger with the needle of a syringe containing *L. ictero-haemorrhagiae*. Towler & Walker (1927) reported cases, but in 1941 Ashe et al., could discover records of only 67 instances and in 1945 Bertucci of only 100 instances in the country until those dates. The infections were distributed widely among the states but predominantly in cities such as New York (Tiffany et al., 1942), San Francisco (Meyer et al., 1939) and New Orleans (Senekjic, 1944; Packchanian, 1938).

It must be admitted that a few of the outbreaks referred to above have been shown to be due not to *L. ictero-haemorrhagiae* but to other leptospiras. For instance, Brown (1928) showed that the strain in the Andaman Islands is the Rachmat type.

In contrast to these countries, there are some where in spite of investigation very little has been revealed. For instance, Egypt has shown no cases up to 1930 (Van Riel, 1939) and very few, if any, since; and in Northern Sudan, Kirk (1938) found no evidence of it. In South Africa, Buchanan (1946) reported that during the previous 20 years search had been made for the disease among 200 jaundiced patients from Transvaal, Orange Free State and S.W. Africa without success. Similarly, although

the disease was suspected in India (Paramand, 1922) and evidence of it had been sought for long by Colonel Taylor (who investigated the endemic form of the leptospiral disease in the Andaman Islands) it is considered that the first proved Indian case was reported in 1937 by Das Gupta and Chopra and the next series of five in 1939 by Konar et al. The varying occurrence of leptospira in the rats in these African countries and India will be referred to below. In China and Russia very few cases have been reported, perhaps for lack of investigation, although Russian workers have done original work on swamp-fever caused by *L. grippotyphosa* (Tarassof 193<sup>1</sup><sub>1</sub> and 1935). Viskovsky (1944) reported an epidemic of jaundice in Leningrad which was leptospiral and probably due to *L. ictero-haemorrhagiae*.



HISTORY OF WEIL'S DISEASE IN GREAT BRITAIN.

In order to give an account of the study of Weil's disease by British workers as well as of the disease as it has shown itself in this country, some detail will be given of the results (mentioned above) of British investigators in France in the First World War. Stokes & Ryle (1916, a & b) diagnosed spirochaetal jaundice on a clinical basis in ten soldiers and demonstrated the spirochaetes reported by the Japanese in guinea-pigs successfully inoculated from two of the ten. They noted that there was no enlargement of the spleen in their patients. Later, Stokes, Ryle & Tytler (1917) reported about 100 cases. They infected guinea-pigs with blood taken from patients from the second to the seventh day of the illness, showed protective antibodies in the serum of convalescents but failed to culture the organism. They demonstrated spirochaetes in guinea-pigs inoculated with material from wild rats. Dawson & Hume (1916-17) gave an account of 178 soldiers suffering from infectious diseases with jaundice. They separated them into 76 with spirochaetal jaundice and 102 which were diagnosed as enteric jaundice or catarrhal jaundice. Of the spirochaetal type (mostly diagnosed clinically) 18 were severe and 58 mild; they found that splenic enlargement was uncommon. They passed the infection to a guinea-pig from at least one patient and saw what they took to be spirochaetes in the blood and urine of some others. Dawson, Hume & Bedson (1917) gave a summary of their work and of that of the Japanese, French and Germans and gave a low case mortality of 4 to 5 per cent in their experience in Belgium.

In Great Britain the first proved infection was of a

man who became infected by immersion in the Thames; the causative organism was found in his blood by guinea-pig inoculation (Manson-Bahr et al., 1922). There followed the reports of Gulland and Buchanan (1924) and Buchanan (1927) on the disease in coal-miners in East Lothian, in a brewery worker, a labourer in a piggery and others, with records of transmission of the infection to guinea-pigs from the urine of two patients and the microscopical finding of forms suspected of being leptospira in the urine of fifteen others (Buchanan, 1927, p.53). Buchanan also demonstrated *L. ictero-haemorrhagiae* in rats in Scotland and in slime from a coal-pit.

During the next ten years few papers on Weil's disease were published in Great Britain. Good clinical accounts (with little bacteriological support) were given by Burton-Fanning and Cleveland (1926) and by Brown and Cleveland (1932). In the first of these reports, four patients in Norfolk were diagnosed clinically as Weil's disease; spirochaetes of doubtful description were seen in the urine of one. The features of the illnesses were sudden attack and severe prostration; jaundice on the 5th or 6th day; haemorrhage from various parts occurring later, with remission of fever; renal symptoms, including suppression of urine in two, albuminuria in two others and haematuria in one of these; three died. One patient had been scratched or bitten on the face by a rat six days before the illness. Brown and Cleveland's patient was a farmer in Norfolk who was taken ill five days after being bitten by a ferret which had itself just before been bitten on the lip by a rat. The ferret died subsequently. The farmer's illness was like Weil's disease clinically; he died and sections of the kidneys showed

spirochaetes by Levaditi's stain.

There was almost certainly <sup>still</sup> some confusion between Weil's disease and infectious jaundice of other causes. This was due to the lack of serological tests by all but a very few (Brown & Davis, 1927) of the bacteriologists interested and to the belief that in guinea-pigs inoculated with material from suspected patients, lung haemorrhages without other lesions and without the demonstration of leptospira in blood or tissues might be taken as partial evidence of transmission of leptospiral infection. In Buchanan's (1927) report on 22 patients, he relies (page 11) on this evidence in eight patients with or without the microscopical demonstration in the patient's urine of the forms which "could only be regarded with suspicion as of the leptospiral type", (page 53). In nine others his pathological evidence of the infection is only these microscopical appearances in the urine with no result of inoculation into guinea-pigs. These facts may explain why eight of his cases were of young people in two families - five in one family and three in another. It has been found later to be a very rare occurrence for more than one infection by *L. icterohaemorrhagiae* to occur in a family at one time. An example, however, is a woman whom I have seen and investigated who developed the disease at almost the same time as her husband, in circumstances unknown for either.

Three examples may be given of small outbreaks of jaundice in which appearances suggestive of leptospiras in urine and (in the second and third reports) haemorrhages in the lungs of guinea-pigs injected with it led to a suggestion that Weil's disease might be the diagnosis. The reports were by Hindle &

Brown (1925) about a school in England, Mackie & Mc.Lachlan (1927) of school children in Ayrshire and Elliott & Beattie (1933) about adults and children in Selkirkshire. In all three outbreaks there were features later realised to be rare in leptospiral jaundice and common in catarrhal jaundice. These are multiple infections among people in contact with one another and a series of cases extending over a period of time with intervals of one month between certain dates of onset. During fifteen years of experience of Weil's disease among sewer workers I have not encountered an infection in a home contact of a sewer worker, and Pickles (1936) showed very clearly the incubation period of one month in catarrhal jaundice.

In 1927, Brown & Davis adapted to leptospira a serological test previously used for trypanosomes, in which contact of organisms and specific antibodies is shown by the adhesion to the organisms of other bacteria or blood-platelets. By this they demonstrated antibodies to *L. ictero-haemorrhagiae* in the blood of wild rats and distinguished from one another *L. biflexa*, *L. hebdomadis* and *L. ictero-haemorrhagiae*.

In 1931, Findlay et al. provided a very useful distinction, by showing that <sup>in</sup> catarrhal jaundice there is a distinct relative increase of mononuclear leucocytes in the blood. In July, 1934, Fairley reported a fatal Weil's infection in a sewer-man in London and serological tests showed that eight other sewer workers who had previously suffered from jaundice, from seven months to twelve and a half years before, had been infected with *L. ictero-haemorrhagiae*.



In 1935 H.C. Brown found that the adhesion test was equal in sensitivity to the agglutination reaction with formalised leptospira which Schüffner (1934) had been using in Holland. This paper and Fairley's led to sera being sent to Brown from all parts of England and Wales with the result that 40 cases were diagnosed in seven months. From that time single cases and large and smaller groups have been found in all parts of the British Isles. The infections recognised since July, 1933, in published and unpublished records have been, from time to time, added together and analysed by occupation. For example, Alston and Brown (1937) thus gathered 142 cases for the period July, 1933 to February, 1937; Alston (for the Third International Congress in Microbiology, 1939, unpublished) increased the number to 274 for July, 1933 to July, 1939; Broom & Alston (1948) added 195 cases which they had themselves diagnosed from the beginning of 1940 to the end of 1946 and Gardner & Wylie (1946) reported finding 182 cases in the years 1940-1945. From these and other sources I have found records approaching one thousand infections with Weil's disease in Great Britain from July, 1933 to 1948 and I have analysed these as regards occupation in Table 1 which is appended.

As regards distribution in different parts of the British Isles, full records are not available but a map which I have prepared (Map I appended) from locations made known to me and to Dr. J.C. Broom (unpublished) of 669 cases between July, 1933 and December, 1946, shows that the disease has been found very widely in the country. The areas in which the greatest numbers of patients have been found are Aberdeen (approximately

200), Glasgow and S.W. Scotland (approximately 70), Northumberland and Durham (approximately 50), London (approximately 100) and Hampshire (40). In each of these areas, to opportunity for infection of patients has been added instructed anticipation of doctors.

Broadly speaking, the disease is diagnosed at the present time in this country in the more severe, icteric form. Broom & Alston (1948) found absence of jaundice in ten per cent of 120 cases in their 1940-1946 series of patients from all parts of England and Wales. In London sewer workers among 40 instances of Weil's disease which I investigated personally between 1934 and 1948 I found three anicteric cases (8 per cent). But quite different conditions are found when heavily infected populations can be observed. Ido and Wani (1917) reported that in an epidemic 60 per cent did not show jaundice and in the British Army in Flanders Stokes & Ryle (1916) had the same experience, as also did Jitta (1935) in Holland. In 1935 I found (Alston & Brown, 1935) agglutinins and protective antibodies in the blood of 9 out of 45 (20 per cent) London sewer workers who were in good health at the time of the test and who said they had never had jaundice. Smith & Davidson (1936) showed that of 51 fish-workers whose serum significantly agglutinated *L. ictero-haemorrhagiae* 36 (70 per cent) could not remember having had jaundice. Among the anicteric infections are those which show meningitis and these will be mentioned later. It seems safe to say that half the cases that receive medical attention either in hospital or at home are not accurately diagnosed.



In June, 1934, Weil's disease was diagnosed on clinical grounds and confirmed serologically in two patients in Aberdeen who worked at handling white fish in unhygienic sheds. Davidson et al. (1934) reported at the end of the year a total of thirteen such infections and six more examples of leptospiral infections in other surroundings. They showed bacteriological or serological confirmation in thirteen of the nineteen cases. They demonstrated *L. ictero-haemorrhagiae* in water on the floor of some of the premises in which the work of filleting, cleaning and packing the fish was done. In the sheds, rats known to be infected with the leptospira infested the premises. They continued the work for several years (Davidson & Smith, 1936; Smith & Davidson, 1936; Davidson & Smith, 1939). They found 98 infections of this kind in 1934-38 and Dr. Smith has recently written to me (unpublished) that the total up to 1946 is approximately 200. As well as full clinical, pathological and biochemical studies they confirmed the basis of diagnostic procedure since, in the first four days of the illness, leptospiras were recovered from the blood in 100 per cent of cases examined, while none were recovered after the eighth day; a positive serological result was obtained in some cases as early as the fourth day and was to be expected by the ninth; and from the urine leptospiras were found with less certainty - in 15 per cent of 173 specimens tested by guinea-pig inoculation. They showed that the disease without the jaundice and infection in a symptomless form were common, that immune bodies were excreted in the urine and that sodium hypochlorite was a disinfectant which might be used in preventive action against the disease. (Smith, (1937) protected guinea-pigs against infection by *L. ictero-haemorrhagiae*

by means of vaccines of avirulent living strains or vaccines of virulent forms killed by different antiseptics. When such vaccines were used for injecting human beings only relatively small amounts of lytic antibodies were produced.

As regards fish workers elsewhere Broom & Alston (1948) include eleven infections in men working at the fish docks of Hull and two at Grimsby; Hampson (1946) found leptospiral antibodies in the serum of only 1 out of 116 fish-cleaners at Grimsby and he attributed this low incidence to the use of sea water in washing the fish and the tubs. I know of other fish workers and fish-mongers infected in Brixham, Isle of Man, Billingsgate Market and London. Jeghers et al. (1935) reported an instance in an American fish-cutter and an infection was found in the fish-market of Melbourne ("Health", Canberra, 1938).

In Glasgow and the South-West of Scotland Dr. R.D. Stuart has, for the last ten years, detected and studied leptospiral infections. His report of Weil's disease in a tripe-dresser (Stuart 1938) was of a woman who had been employed for eight years as a tripe scraper in the corporation meat market of Glasgow. The infection was proved by recovery of the leptospira from the urine and by positive serological tests. Tests on the serum of 25 other women employed either continuously or with intervals of absence showed 4 which gave reactions which were considered significant of former infection. The premises were well built and cleaned by hosing with water after the day's work but it was well known that large numbers of rats came to the tripery at night. Leptospira were demonstrated in rats caught in the premises. These infections in tripe dressers are "special cases", as it were, of infections in slaughter-houses (Schüffner 1934).

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undertakings in both regions; rats, they believed, gained access to the workings via level or sloping roadways entering mines or possibly with horse-feed carried down a mine shaft. In 1935 & 1938, Swan and McKeon reported 27 instances of the disease among miners in the area of Newcastle-on-Tyne and Broom & Alston (1948) added 11 more from Northumberland and Durham. A few cases have been reported from miners in other districts.

Infection of men and a few women serving in H.M. and Allied Forces in the British Isles occurred chiefly during the Second World War. The Army and A.T.S. supplied most cases, due<sup>to</sup> rat-infested dwelling quarters and camp-sites and to exposure to stagnant water on manoeuvres. In Table 1 some of the 67 attributed to the Army contracted the infection overseas. Four examples of the disease were found in Italian prisoners-of-war, and two among German prisoners. The Navy provided 19 including 12 found in the Free French sailors living in rat-infested boats at Cowes (Gauld, 1947). Eleven patients in Table 1 were contributed by the Royal Air Force.

Table 1 records 48 examples of infection due to bathing or to sudden immersion in fresh water; seven are known to have been by sudden immersion including one example of attempted suicide. The largest single group of these cases occurred in rivers in Hampshire (Robertson, 1946) but rivers, canals and lakes in many counties of England have contributed cases. Three soldiers were infected by bathing in the Basingstoke Canal (Coles 1936). I know of two children who stated that they had paddled but not bathed. Mention will be made later of two brothers infected in the River Stour in Kent, one who showed mild



meningitis and the other an anicteric form of the disease, simulating appendicitis.

Men working in fresh water comprise 16 in Table 1 .

The work was in water-cress beds, gravel pits, canals or rivers.

The nine infections due to bites of rat, dog or ferret will be referred to in more detail in connection with carriers and modes of transmission of the disease.

From 1939 to 1946 Professor A.D. Gardner collected cases of Weil's disease in which he had provided confirmatory diagnosis by agglutination test. As noted, he and Wylie (1946) analysed and reported these. Gardner (1943) suggested that he had isolated from a dog a new strain of leptospira; the proof of this was not conclusive.

During the Second World War leptospiral infections were found among British troops in Western Europe. Buckland & Stuart (1945) thus reported 33 infections with *L. ictero-haemorrhagiae*, 2 with *L. grippo-typhosa* and 1 with *L. sejroe*. Thirty-nine cases of Weil's disease in Normandy reported by Bulmer (1945) probably include some of Buckland & Stuart's series.

A few infections contracted by Allied troops in Normandy and Italy are mentioned by Broom & Alston (1948) and infections contracted in the British Isles by British, United States and Free French servicemen are included in Table 1 .

My own work with sewermen and others is described more fully in a later section of this thesis. Apart from the sewer workers to be mentioned there these workers have been infected in Glasgow, Aberdeen, Newcastle, Wolverhampton and Brixham.

In summary, the initiative in seeking for and studying

leptospirosis infections in this country has been chiefly with a number of bacteriologists. These have included Adrian Stokes, and S.P. Bedson, in France and Belgium during the First World War, H.C. Brown and J.C. Broom, George Buchanan, John Smith, R.D. Stuart and me who began an interest between the two wars, and A.D. Gardner and F.E. Buckland who entered the field during the Second World War. Of the clinicians who have systematically studied the disease, Ryle and Dawson have been noted, L.S.P. Davidson has been mentioned and K.M. Robertson (1938 & 1946) should be included. This last named clinician has reported 40 cases of Weil's disease in Hampshire in boys and men who bathed or worked, as in water-cress beds, in various streams; he made very good observation on prognosis, treatment by antileptospirosis serum, leptospirosis meningitis and other matters. His diagnoses were confirmed serologically by J.C. Broom.



THE CARRIERS OF THE INFECTIVE AGENT.

The spread of Weil's disease is almost entirely due to the fact that some species of small rodents and a few other animals harbour the organism for a shorter or longer time and excrete it in the urine. By far the most important species are certain rats. This was realised by Japanese workers in 1914 and has been quoted already and was confirmed, as noted above, by German and British workers on the Western Front and by Noguchi in the U.S.A. Since the end of the First World War, leptospiras have been found in wild and domestic animals in almost all parts of the world. The report about rats of different species are very numerous but vary in the methods used in identification of the type of leptospira and in determination of pathogenicity for guinea-pigs. Larson (1943), in a survey of rats in and near Washington D.C., found that for detecting *L. ictero-haemorrhagiae*, agglutination tests of blood were nearly twice as revealing of infection as inoculation of guinea-pigs or mice with emulsions from rats' kidneys; but, of course, the agglutination test almost certainly exaggerates the number of rats which excrete living leptospiras at any time and equally, therefore, the risk of infection to man and other animals. In almost all of the countries mentioned above as sites of Weil's disease, rats have been found to be infected and only a few specially authoritative examples and some from Great Britain need be mentioned. Ido, Hoki, et al. (1917) found virulent leptospiras in the kidneys of 40 per cent of *Mus Decumanus* and of only 0.8 per cent of the *Mus Alexandrinus* and they quote the findings of Miyajima that the field mouse, *Microtus montebelloi* harbours the organism frequently.

Coles (1918) is credited with the first discovery of the organism in rats in Great Britain; he found leptospira by dark-ground illumination and stained films, in 9 out of 100 rats in and around Bournemouth and Stevenson<sup>had</sup> found infestation of rats in two series by 1922 (Stevenson, 1922; Balfour, 1922). In Scotland, Buchanan (1927) reported 37 per cent of 166 rats and one field mouse infested; in over half of the rats considered positive, infection was conveyed to a guinea-pig from kidneys of individual rats. Other examples have been mentioned in the work of Davidson and Smith in Aberdeen, of Stuart in Glasgow, of Brown and Alston in London. A table is given of references to some of the more extensive surveys made of rats and other rodents (Table 2). A very full list is given of these by Walch-Sorgdrager (1939). For the reasons already given the correlation of individual types of leptospiros and their animal hosts and the countries of their occurrence is not sufficiently clear. In Great Britain, only *L. ictero-haemorrhagiae* and *L. canicola* have yet been found in animals or in human infections. Among rodents in the British Isles, apart from rats, field mice have occasionally been found infected, (Buchanan, 1927)<sup>Elton (1931)</sup> reported that 8 per cent of 356 field mice (*Apodemus sylvaticus*) harboured leptospiros but H.C. Brown (unpublished) failed in 1936 to find leptospiros or antibodies to *L. ictero-haemorrhagiae* in 36 field mice (*Apodemus sylvaticus*) from Oxford and injection of macerated tissues of these mice did not cause any disturbance in guinea-pigs.

The relative infrequency of Weil's disease in India and Egypt have been already noted. In Bombay, Colonel Anderson

(unpublished) examined 100 of each of three species of rats for leptospiras and found them in 32 per cent of *R. norvegicus*, 7 per cent of *Gunomys varius* and none of *R. rattus*. These leptospiras were not identified as to type. In Northern Sudan, Kirk (1938), examined 259 rats (*R. rattus*, *R. norvegicus* and *Arvicanthus testicularis*) and by dark-ground illumination of fresh specimens, by guinea-pig inoculation and by agglutination tests he failed to find leptospiral infection. He considered that climatic factors, especially the strong sunlight, might contribute to this.

Examples of naturally occurring infections of white rats in laboratories (Korthof, 1937 and Roelcke, 1938) and of a guinea-pig recently obtained from an animal dealer (Mason, 1937) are known. Young rats are less infected than old ones (Scüffner, 1934) but the infection causes only slight disturbance.

Although *L. ictero-haemorrhagiae* and *L. canicola* are the only leptospiras which have yet been identified in natural circumstances in the British Isles it is interesting that several muridae which have been found in other parts of the world to harbour other leptospiras than the two mentioned. I have compiled ~~which occur here~~ a list of British muridae from M.A.C. Hinton's book, "Rats, Mice as Enemies of Mankind" (1918) and against the appropriate names placed the species of leptospiras found in other, especially European, countries. I drew the information of host relationships from papers of Rimpau (1943) and Petersen (1944a, b, c).

British MuridaeLeptospiras.Microtinae (voles & lemmings)

Evotomys glareolus

L. grippo-typhosa

Microtus hirtus

Microtus agrestis

Arvicola amphibius amphibius

" " reta

Murinae (mice & rats)

Apodemus sylvaticus

(L. grippo-typhosa  
L. sejroe  
L. bataviae)

" flavicollis wintoni

Micromys minutus

L. bataviae

Rattus rattus

Rattus norvegicus

L. pomona

Mus musculus

Thus, four species of leptospiras unknown in nature in this country occur abroad in muridae which are to be found in various parts of the British Isles. Investigation of the possibilities is needed.

In addition to the hosts and parasites in the list given above, muridae closely related to some named have been found to harbour leptospiras, viz. Apodemus flavicollis harbouring L. sejroe and L. saxkoebing, and Mus musculus spicilegus with L. ballum and L. saxkoebing (Petersen 1944, a,b,c).

It is interesting that the species of leptospiras reported in Apodemus sylvaticus by Elton et al. (1931) were found by A.D. Gardner to be only very feebly pathogenic to guinea-pigs.

Next to rodents, the dog family suffers infection with L. ictero-haemorrhagiae. Leaving aside L. canicola, after it was distinguished in 1931 from L. ictero-haemorrhagiae,



(Klarenbeek and Schüffner, 1933), leptospiral disease in dogs was first reported in France by Courmont and Durand (1917); it was thoroughly investigated in Great Britain by Okell, Dalling and Pugh (1925), who found that most cases were in the country districts among sporting dogs and the case mortality was 95 per cent. Dogs suffer, with or without jaundice, from infection by *L. ictero-haemorrhagiae* and may also be either symptomless or convalescent carriers. Klarenbeek (1938) reported 57 instances of infection by *L. ictero-haemorrhagiae* among dogs at Utrecht between 1933 and 1937; nearly one half of these illnesses occurred in the months of September, October and November and the same fraction of the whole ended fatally. Petersen and Jacobsen (1937) found that the serum of 18 out of 53 dogs in a Danish village showed agglutination of *L. ictero-haemorrhagiae* in dilutions of at least 1 in 300; they believed that infection passes from dog to dog as well as from rat to dog and that the infection was entirely symptomless in most of the sero-positive animals. In Germany, Uhlenhuth and Zimmermann (1936) found infections, mostly latent, in about 6 per cent of 90 dogs. Dogs excrete the organism in the urine for varying times and the chance of infection being demonstrated increases with the age of the animals (Van der Walle, 1939). Some surveys of infections in dogs in this and other countries are recorded in Table 3. It appears that, so far as is known, dogs of all breeds are susceptible (Meyer et al., 1939).

It may be noted here that very few human infections have been traced to dogs. Krumbein and Fieling (1916) reported two cases (which were not proved bacteriologically) which they very credibly related to a dog which was ill with jaundice and



haemorrhages. This dog belonged to a German Army officer who was the first of the two patients. He scratched his hand on the dog's tooth while cleaning the animal's mouth during its illness. Eighteen days later he himself was taken ill with an illness which, from the description, had all the features of Weil's disease; he recovered. An Army doctor who had been in contact with the first patient and the dog became ill 19 days later with Weil's disease. In 1936, Jacobsen described a few such cases in Denmark.

Passive transfer of the infection by dogs is recognised, such as the disease in a soldier groom who was bitten by a dog which had just killed a rat (Wigmore and Denning, 1936). There is evidence that dogs in kennels are more infected with *L. ictero-haemorrhagiae* than house dogs (Stuart, 1946) but infection and the carrier state with this organism are well established in dogs in general in this and other countries (Uhlenhuth et al., 1936; Petersen et al., 1937; Meyer et al., 1939).

In view of this, infection of kennel-men is unexpectedly uncommon; I have known one case in an attendant at a dog-racing track, where jaundice had occurred among the dogs and where rats were known to be present. Also, infections of women or children in the home (such as occurs with *L. canicola*) are almost unknown. It may be relevant that Petersen (1944) found that only a small proportion of sero-positive dogs in Denmark secrete *L. ictero-haemorrhagiae* in the urine.

Dunkin and Laidlaw (1924-25) proved that a wild fox which was found sick and jaundiced in an open field near a research laboratory was infected with *L. ictero-haemorrhagiae*; and Dr. J. Smith has written to me that he investigated an out-

break of leptospiral jaundice in a silver fox farm near Aberdeen and that three foxes died of the disease.

Infection of pigs is known. Sander (1935) recorded an instance of proved Weil's disease in a butcher who, eight days before his illness, killed a very jaundiced pig. Klarenbeek and Winsser (1937) proved by serological tests and by guinea-pig injection that a spontaneous infection in a severely jaundiced pig was due to *L. ictero-haemorrhagiae*. Johnson (1943) quoted that an endemic state of pigs heavily infected with *L. ictero-haemorrhagiae* had been found in 1940 in Western Samoa. Roch and Mach (1947) found in several pigs agglutinins for *L. ictero-haemorrhagiae* at higher dilutions than for any other type of leptospiras which they tested. (Table 3).

In 1943, Kathe reported that he had found serological evidence of infection of two horses and two cows with *L. ictero-haemorrhagiae*; Petersen (1944) quotes Ottosen as finding antibodies to *L. ictero-haemorrhagiae* in a cat with jaundice.

With less evidence as to accuracy of type of leptospira, Bertucci (1945) adds to the list of animals mongooses, minks, bats, poultry and bandicoots.

VIABILITY OF L. ICTERO-HAEMORRHAGIAE OUTSIDE THE ANIMAL BODY.

The most important source of infection of the human race by *L. ictero-haemorrhagiae* is the urine of rats and the viability of the leptospiras after they have been excreted on soil or into water is very important.

Zuelzer (1928) showed that the organisms are, in nature, usually found attached to other spirochaetes and protozoa.

Noguchi (1918c) stated that in contaminated waters they were not capable of multiplying, and survived less than 48 hours, and when added to distilled water they disappeared in seven days. They were found in considerable numbers in the slime of the roof of a coal-mine (Buchanan, 1927) and in floor-washings from fish-cleaning sheds (Davidson et al. 1934). In 1934, Alston (1935)

isolated pathogenic *L. ictero-haemorrhagiae* from a specimen of slime from the floor of a London sewer at the edge of the stream and from the outlet of a house-drain into a different sewer.

Twenty-eight similar specimens of slime from the floor or lower parts of the walls of sewers or from outlets of drains produced no sign of infection in the animals inoculated with them and six specimens of the small, slimy stalactites which form on the roof did not give rise to any infection in guinea-pigs. It is known that rats are very numerous in the sewers at night and the small proportion of recoveries of *L. ictero-haemorrhagiae* is partly due to the presence of other living organisms, because the reaction of the mud is amphoteric or slightly alkaline which is in itself rather favourable. In 1936 (unpublished) I found that leptospira in sewer-mud may remain virulent to guinea-pigs for as long as twenty-four hours after the mud has been brought to the laboratory. A view differing from that of other workers is given by Van Thiel

(1937) who found that *L. ictero-haemorrhagiae* remained virulent in water for 22 days and tended to sink to the lower levels.

The organism is very susceptible to the action of acids and is rarely found in waters having a pH less than 6.8. For instance, Taylor and Goyle (1931) reported that in the Andaman Islands, leptospira were common in waters of pH 6.9 or over and absent in those of 6.6 or less and Sardjito and Zuelzer (1929) have shown that they are abundant in the alkaline waters of Sumatra where human infections (due to *L. ictero-haemorrhagiae* and other species of leptospiras) are frequent, but are practically absent from the more acid waters of Java, where there are few infections.

*L. ictero-haemorrhagiae* is susceptible to common salt and it cannot live for more than three days in alkaline water containing 0.17 per cent or more of sodium chloride; it perishes rapidly in sea-water. Schüffner considers that these facts provide part of the explanation of the variations in incidence of infection by immersion in water in different parts of Holland since there is a mixture of sea and fresh water in different proportions in different parts of the country. In Amsterdam, in the summer of 1944, (Ruys, 1946) there were no instances of Weil's disease due to bathing or sudden immersion and this is attributed to the fact that in those months the Germans flooded the canals with sea-water so that in one particular canal the sodium chloride rose in August to 1572 mg. Cl per litre, contrasted with 639 to 1045 in other years.

Among artificial anti-leptospiral agents, Davidson and Smith (1936) showed that sodium hypochlorite in a concentration of 1 in 4,000 or greater killed *L. ictero-haemorrhagiae* in five

minutes. In 1937 I (unpublished) found that in sewer mud virulent *L. ictero-haemorrhagiae* survived exposure to an ultra-violet lamp at 18 inches distance for five minutes, but were killed in 10 minutes.

It has long been recognised that the organisms die rapidly when they are excreted in the urine of patients. It has been considered that the acid state of the urine, which is increased in the febrile condition, is responsible for this and for purposes of cultivating the leptospira or transmitting the disease to experimental animals, a practice is made of rendering the urine alkaline as soon as possible after it has been passed, or of giving the patient alkalis in order to alter the reaction in vivo. In 1936, Davidson and Smith brought evidence that lytic antibodies which are present in the urine in some cases may have on the leptospiras an additional lethal effect, commencing as soon as the organisms reach the renal tubules. Independently, Van der Hoeden (1936) demonstrated specific agglutinins and lysins in the urine of men, dogs and wild rats following a reaction to infection.

It is interesting that Brown and Cleveland (1932) reported that it was a common practice with some rat-catchers to urinate immediately on the wound of a ferret which had been bitten by a rat because they believed that this was the best thing to do to prevent the ferret from dying. Mason (1938) found that six out of eleven rat-catchers had antibodies to *L. ictero-haemorrhagiae* in the blood without previous jaundice, and Van der Hoeden (1936) and others have shown that antibodies in the urine often accompany them in the blood. Professional rat-catchers might be expected to provide several examples of Weil's disease, but I know of only two examples in this country. It may be that experienced rat-



catchers urinate not only on rat-bites of their ferrets but on their own bites also. One rat-catcher whom I knew may have increased his immunity by his occupational side-line of biting off the heads of rats for a small reward outside public houses.

### THE ROUTE OF INFECTION.

Weil (1886) believed that infection took place through the alimentary canal. Inada et al. (1916) were at first of the same opinion, but later they were satisfied that infection could take place through the shaven but macroscopically unbroken skin of a guinea-pig and, although they were able to infect animals through the mucous membrane of the alimentary canal, massive doses of infected liver tissues were used for the purpose. In studying 55 cases among coal-miners, these observers obtained only a few instances suggesting cutaneous origin, but they stated the cutaneous route was the probable means of entry because of the following facts: (1) The incidence was greater in certain parts of the coal-mine than in others, (2) there are many cases in wet and few in dry mines, (3) the infection takes place more easily if the skin is injured, (4) coal miners are liable to abrasions of the skin and also to skin lesions caused by working with the feet in water.

In general, experience confirms that the skin - especially through cuts, abrasions, bites or sodden surfaces - is the usual site of entry. Welcker (1938) collected from the literature a very instructive group of 25 examples of accidental leptospiral infections (including 23 of Weil's disease) in laboratories. Of those twelve in whom the site of entry was considered accurately observed, it was piercing of the skin by an infected syringe needle or broken glass tube in four, by bite of an infected rat in three, by way of the conjunctival sac in three, by contamination of the face (perhaps including the eye) in one and by getting infected urine into the mouth from a pipette in one. Of these, the

infections by conjunctiva and by mouth are especially interesting because they may be relevant to some infections during swimming or sudden immersion in fresh water. Varvello (1940) experimented with infection by *L. mitis* (syn. *L. bataviae*) in human volunteers and produced infection by immersing a leg for two hours in water containing culture; he was unsuccessful when a hand was so immersed for one hour and when drops of dilute culture were instilled in the eye or when the mouth was repeatedly rinsed with dilute culture; success after passing culture to the stomach by tube may have been due to abrasions of the nasal mucous membrane.

Regarding the incidence in sewer workers in London I found a difference between the two classes of workmen involved. Both classes of men are subject to abrasions of the hands and arms but the builder's labourers who break up and handle old brick work or concrete covered with slime (photographs 1 & 2) suffer more abrasions than the "flushers" who clean the walls of the sewers, and I found that the builder's men during the eighteen months in 1934-1935 showed a case rate ten times as high as the flushers. Among sewer workers I saw several patients with Weil's disease who showed gashed and abraded wounds when they were admitted to hospital, and these wounds had been made at a time within the incubation period of the disease.

Further evidence of the cutaneous route of infection is given by the incidence of the disease in fish-workers (Smith and Davidson, 1936) and in sugar-cane cutters in Queensland (Cotter, 1936). Examples of infection by needles or syringes have been given already and (Cattaneo, 1929) gave a well-proved instance of the disease with onset two days after the patient had trodden

with an abraded foot in the blood of a rat which had just been killed. **Rat scratches have infected**(Evans et al. 1946).

Infection by rat-bite was recognised by Ido et al.(1916), who observed two patients who acquired Weil's disease seven days and from eight to nine days respectively after rat-bites. I knew three similar instances in this country during 1935 to 1937 and in two of them the illness began between nine and fourteen days after the bites, which is within the incubation period.

The question whether leptospira can penetrate the unbroken skin may be held somewhat academic on account of the microscopic size of the abrasion which might well serve as portal of entrance for a leptospira, but Inada et al. (1916) believed that the organism could penetrate the unbroken skin although they obtained a higher percentage of infections through an abraded surface. Again Welcker's (1938) series of laboratory infections gives some evidence, because some of them occurred through macroscopically intact skin, unless efforts at disinfection after the accident damaged the surface without killing the leptospiras for, in some instances, such disinfectant treatment failed to prevent the disease.

Wet or sodden skin is, without obvious injury, an aid to the penetration by leptospiras (Petersen, 1944). Infections through the feet by treading on infected ground beside streams or ponds after bathing in fresh water share in different cases the accessory elements of wetting and abrasion of the skin. Esseveld (1937) has shown that by similar circumstances, angling carries a risk of infection.

Regarding the oral route of entrance it is well known

that by "water accidents" with sudden, accidental immersion in water, infection is more likely to occur than in the course of ordinary bathing. The first proved case of the disease in this country was due to falling into the Thames at Gravesend (Manson-Bahr et al., 1922) and Schüffner (1934), Ruys (1946) and other Dutch workers have studied this type of infection extensively. The special danger of it is, no doubt, that an unpremeditated immersion often causes violent struggling so that water enters the respiratory and alimentary tracts. In swimming in infected water there is a risk, of uncertain degree, of conjunctival infection and Schüffner (1934) drew attention to the danger of the "crawl" stroke in allowing infected water to enter the mouth readily. It has been suggested that leptospiral meningitis due to *L. ictero-haemorrhagiae* may be particularly associated with bathing because the route of entry is the fauces or conjunctiva (Buzzard and Wylie, 1947).

When the organism enters by the mouth, invasion of any part of the alimentary tract by leptospira is possible as an additional or alternative means, but indisputable evidence of alimentary infection is rare. Contamination of food by rats' urine has been suspected, and Jorge (1932) described an outbreak in Lisbon with 126 cases in a month, which he attributed to drinking water from a certain fountain that had been fouled by rats. Although the organism was not isolated from any of these cases and guinea-pigs were injected but did not become infected, the serum of several patients was found to agglutinate both the London and the Lisbon strains of *L. ictero-haemorrhagiae*. It is a pity that the cause of this outbreak was not conclusively proved, because, if it were indeed an epidemic of Weil's disease,



it could provide some interesting epidemiological evidence.

Martin (1938) considered that among coal-miners of the Gard, in S.E. France, contamination of food and drink and of underground water which the miners drink is an important means of infection.

Mechanical transmission of the infection sometimes occurs. Reference has been made to the case in which Major H.C. Brown detected the disease by agglutination in a patient who had been bitten by a dog which had just previously killed a rat (Wigmore and Denning, 1936) and to the case reported by Brown and Cleveland (1932) in which infection followed a bite by a ferret which had immediately before been bitten on the lip by a rat. In these cases it is only natural to assume that the teeth of the dog and of the ferret were contaminated by contact with the infected viscera of the rats. Alston and Brown (1937) knew another example of ferret bite in which the same mechanical transmission of infection is probable, since they could not demonstrate agglutinins in the ferret's blood. In these three cases the intervals between the bite and the onset of the illness were five, seven and ten days respectively which agree with the ordinary incubation period and resemble that in those infections developing after rat-bite, which have been mentioned.

During the early investigations of the disease it was suspected that insects might provide a means of transmission of the infection, either mechanically or as intermediary hosts of the leptospira, but this has not been confirmed in any form. Noguchi reported in 1918 that the larvae and adults of the *Culex* mosquito, the larvae of the house fly and the blue-bottle fly, wood-ticks (*Dermacentor andersonii*) and leeches failed to become carriers of the leptospira when fed on infected guinea-pigs or

their organs, and that therefore they cannot be shown to play the part of an intermediary host of *L. ictero-haemorrhagiae*. Gay and Sellards (1927) found that virulent strains of leptospira survived in *Aedes aegypti* for as long as three weeks in certain instances, but they failed to transmit infection by bites of these infected insects. Bonne (1924) found survival for at least forty-eight hours in the bed-bug, but there is no evidence that this insect plays a part in transmitting the disease. Even in the tropics well-considered opinion is against insect transmission, as is stated, for example, by Taylor and Goyle (1931) with regard to the disease in the Andaman Islands.

Stavitsky (1945 a & b) has studied the routes of infection with *L. ictero-haemorrhagiae* experimentally in guinea-pigs, rats and mice. He found that the routes which led to infection included the surface of injured skin, the mouth, the conjunctiva, but not through the surface of intact skin or nasal mucous membrane; he found the intact conjunctiva one of the most sensitive areas of the body. He showed that leptospira leave the area of intradermal inoculation within 30 minutes of injection; that there was no evidence of phagocytosis of the organisms by leucocytes of any sort and he believed that lytic antibodies play a big part in destroying the organisms in a resistant host.

Schüffner (1934) quotes Doelman as authority for a case in which it seemed likely that a woman had been infected through coitus with her husband when he was recovering from an attack of Weil's disease which had begun 18 days earlier. I have seen a woman in whom Weil's disease began at almost the same time as her husband; The origin of the infection in either spouse was not discovered. Stavitsky (1945a) found that experimental animals may be infected by intravaginal inoculation.

## INCIDENCE AS REGARDS SEX, AGE AND SEASON.

### Incidence as Regards Sex.

Males are much oftener infected than females. For instance, Broom and I found that of 189 cases in which we proved Weil's disease during the seven years 1940 to 1946, only 8 (4.4 per cent) were female (Broom and Alston, 1948). The reason for this inequality is that in spite of half a century of equalisation of the sexes, men are predominant in occupations carried out in rat-infested places. An approximate equality of susceptibility to Weil's disease is shown when men and women work together in such circumstances as cleaning and packing fish in Aberdeen (Davidson and Smith, 1936) or where women work alone in tripe-dressing in Glasgow (Stuart, 1938).

In the British Isles, from published and unpublished sources, I have found only approximately 127 instances of infections in girls and women. About 104 of these have been among Aberdeen fish-workers, 5 in tripe-workers in Glasgow; 10 were published by Gardner and Wylie (1946) and Broom and Alston (1948) found 8 females with infections in the following circumstances:- farm work (2), school-girls with unknown cause (2), hospital nursing, NAAFI, school-girl bathing, wife infected at same time as husband but cause unknown, one each.

From bathing and sudden immersion, only a very small proportion of cases is among girls and women. Reasons for this are, no doubt, that women are not so likely to be suddenly immersed, are less likely to swim in dirty water, to use the crawl stroke or to be careless in running about barefoot on the wet ground at the edge of the water.

Comment has already been made on the surprising rarity of domestic infections of men, women or children from dogs.

Although I cannot trace a single instance of the death of a woman or girl in the British Isles due to Weil's disease, the seriousness of the infection is probably approximately equal in the sexes, when age is taken into account. In Davidson and Smith's ~~series~~ (1939) series of 104 infections (all but six in fish-workers), six deaths occurred among fifty men and no deaths among fifty-four women, but this was considered due to the high incidence of healthy young girls in the fish-trade and to the fact that after the age of 40 when 5 of the 6 deaths occurred, all the employees except one were men.

#### Incidence as Regards Age.

It is chiefly in bathing and in the unusual instances of domestic infection that children are at risk, but a case occurred (Kerr, 1936) of a boy, 10 years of age, who lived in a rat infested house and had bathed in a canal. The occurrence of leptospiral meningitis in children has been emphasised by French writers (Marie and Gabriel, 1936). Robertson (1946) found a range of age from 5 to 78 years in 30 patients infected by bathing and working in water in Hampshire. Broom and Alston (1948) analysed the ages of 100 patients, whom they had diagnosed, in age groups of fifteen years and the distribution varied between 5 per cent of all patients over 60 years of age and 31 per cent in each of the groups 16 - 30 years and 31 - 45 years. (See Table 4). Stewart et al. (1944) saw infection in a child of 4 years

#### Seasonal Incidence.

Seasonal variation of incidence was first noticed in the case of field workers. In Japan it was found that in coal-



mines infection occurred at all times of the year, but in the fields the disease was seen most in the seasons of moderate temperature and least during the hottest and coldest parts of the year. In Denmark, Petersen (1944) found about  $3\frac{1}{2}$  times as many infections in the second half of the year, compared with the first half, on the monthly figures of laboratory diagnosis for the years 1934 - 1943 inclusive; October was the peak month; and many would be diagnoses of infections which had occurred up to a month previously. Bathing is an obvious cause of higher figures in the summer and hot autumns, such as the autumn of 1947 in England and Wales. Schüffner (1934) found a higher incidence in Amsterdam and in Dordrecht during July to October compared with the rest of the year, during the period 1924 - 1933, and when he analysed the cases at Dordrecht he found that the increase during the second half of the year referred to infections due to occupation and unknown causes as well as to swimming and "water accidents".

I find (unpublished) that fifty infections in sewer-workers in London during the years 1934 to 1945 inclusive, show an obviously uneven distribution between the months. In December, January, February and March of all these years there occurred only 6 of the cases and in the remaining two-thirds of the years 44 (88 per cent). These findings are recorded in Table 5. Sewer work in all its forms continues regularly all the year round.

Davidson and Smith (1939) stated that among 98 infections in fish-workers the disease occurred least often in February and March and most often in August and September, although the fish-trade operated at about the same activity throughout the year.

The reason for this concentration of human infections, apart from bathing, in the second half of the year is not clear,



but Klarenbeek (1938) reported that half of 57 dogs which were found infected with *L. ictero-haemorrhagiae* from 1933 to 1937 showed their infection in September, October or November. It may be that rats excrete leptospira more profusely in the second half of the year, but if so, I have not seen it demonstrated. Also, however, in the wetter part of the year, rats may obtain water more readily and not require to invade damp premises so frequently; this might apply, for example, to sewers where it is considered that the rats live and gain food in buildings, and reach the sewers by way of broken drain-pipes in order to drink. (Table 14).

INCUBATION TIME.

The period of incubation has been most accurately recorded by Schüffner, (1934) who found that in 37 cases due to falling into infected water it varied from four to nineteen days and that in 31 (84 per cent) of them it was from seven to thirteen days. In the series of laboratory infections quoted by Welcker (1938), incubation periods mentioned were 2-3, 4, 6, 7, 8 (four times), 9, 10, and 11 days. Infections due to bites by animals give reliable evidence of incubation time and examples are of rat-bites followed by the disease after 7 days (Bie, 1939), 7 days, 8 days, (Ido et al., 1916), 9 days, 14 days (Alston and Brown, 1937); the bite of a dog which had just killed a rat was followed by the disease in 7 days (Wigmore and Denning, 1936) and the bites of ferrets have preceded the disease by 5, 7 or 10 days (Alston and Brown, 1937). Instances of very short incubation periods include a doctor in Welcker's series (mentioned above) who was taken ill 2 - 3 days after squirting into his eye some infected liver which he was grinding, and the first case proved bacteriologically in the British Isles - a seaman who became ill 3 days after falling into the Thames (Manson-Bahr et al., 1922). Two more instances of incubation lasting 2 days are given by Cattaneo (1929) and Fabino et al., (1938).

FATALITY RATES.

In view of what is known of mild and symptomless infections, it is evident that case-fatality rates for different groups will depend on the extent to which the less severe infections are taken into account. Jitta (1935) stated that among more than 450 infections of Weil's disease in Holland in 1932-34 there was not a fatal case which did not show jaundice; this is the experience of all who know the disease and includes reference to those patients who develop meningitis without jaundice. The extent to which anicteric cases are included explains, in a large measure, the difference in case fatality rates of from 5 per cent to 50 per cent. In the future, when the total incidence of the disease will probably be more fully determined, it will be possible to discern more accurately whether some strains of *L. ictero-haemorrhagiae* are more virulent than others, whether some people are more susceptible than others to the same strains or whether some circumstances of infection increase the severity of the disease. At present, the part played by such factors cannot be clearly defined and an average fatality rate of 15 to 50 per cent among patients who have been treated in hospital indicates the risk of a fatal issue in any case with observable jaundice. When I collected records of 142 patients (almost all with jaundice) who were treated in the British Isles between July, 1933 and February, 1937, I found a case fatality rate of 15 per cent, (Alston and Brown, 1937). Similarly (Broom and Alston, 1948), 114 patients (90 per cent with jaundice) in the British Isles during the seven years 1940 to 1946 inclusive, showed a case fatality rate of 22 per cent (Table 6). In Table 13 records of

case fatality rates from different countries have been collated with the percentage which were jaundiced, where that has been recorded.

Apart from jaundice one of the clearest factors is age. This is shown in Table 6 where the case fatality is approximately 16 per cent in each of the three age groups up to 45 years of age and rises steeply afterwards. This is the general experience of Davidson and Smith (1939), although their inclusive death rate was only 6 per cent, for five of the six deaths which they met in 104 infections ~~which~~ occurred after the age of 40.

The absence of jaundice distinguishes the mild or trivial infections from the more serious ones, and the degree of acute nephritis is the best single pointer in the prognosis of the more serious cases. The evidence of serious renal disease is in suppression of urine, coma, greater amounts of blood, casts and albumin in the urine and, as a very important single indication, the concentration of urea in the blood. I have prepared a table (No. 7) of blood urea determinations (in mgm. per 100 c.c.) recorded in some cases of the series published by Broom and Alston (1948) and in some of my own cases before 1940. This table shows the highest recorded figures in <sup>9</sup> fatal cases were 153, 234, 245, 320, 340, 480, 552, 701 and 750 mgm. respectively, and of 22 non-fatal cases the ranges of highest figures recorded during the illnesses was from 33 to <sup>366</sup>~~300~~ mgm. with 11 of these 22 below 150 mgm. From such experience as this I have for some time regarded the figure of 350 mgm. of blood urea per 100 c.c. as critical. Some figures are given in Table 7 of successive determinations in the same patient and they show how rapidly the concentration can rise or fall at intervals of one or a few days.

MENINGITIS DUE TO L. ICTERO-HAEMORRHAGIAE.

In Weil's disease the occurrence of such neurological signs and symptoms as headache, mental excitement or confusion and coma are common accompaniments of severe infections. They are regarded as part of the directly and indirectly toxic effects of the infection on the central nervous system. In contrast, there have been recorded since the beginning of modern study of the disease during the First World War, instances of meningitis without sufficient hepatic damage to cause jaundice. This type of the disease was studied earlier and more thoroughly on the Continent than in Great Britain and an epidemiological problem has arisen, - whether meningitis is more likely to follow certain modes of infection than others. For these reasons, a synopsis of is made of the reports from abroad and an account of experience, including my own, in this country.

French workers were the first to emphasise the meningitic form of the disease. Costa and Troisier (1916) found it among French soldiers and transmitted the infection to guinea-pigs from human cerebro-spinal fluid. They and others made a series of studies and, in 1933, Troisier and Boquien summarised the experience up to that time. They found 20 cases of leptospiral meningitis in the literature; 13 of them were of "pure" meningitis and two of these were due to bathing. They emphasised the features of a sharp onset, muscle pains, ~~headaches~~, epigastric pains and, in nearly half the cases, headache; oliguria was frequent with albuminuria in 40 per cent; some showed slight icterus demonstrating a link with the usual form of the disease. Single or multiple relapses occurred; all the patients, who included children and adolescents, recovered. The cerebro-spinal fluid was



usually crystal clear, showed an increase of cells by the 5th to 14th day of 200 to 300 per c.mm., rising in some as high as 1,330; the majority of these cells were likely to be polymorphonuclear at first and mononuclear later. Protein in the fluid was found in normal values of 30 or 40 mgms. per 100 c.c., rising to 1,330 mgms; the glucose was normal or increased. In the blood, an increase of polymorphonuclear leucocytes was found.

In 1934, Erber confirmed that all cases of leptospiral meningitis in France had recovered.

In 1936, Schüffner and Walch-Sorgdrager found 25 cases of meningitis of which 17 were "pure" meningitis, among 312 infection with *L. ictero-haemorrhagiae*.

Marie and Gabriel (1936), reported meningitis in three children aged 10, 10 and 13 years. They pointed out that in children the meningitic form is commoner than other forms and that epidemic and seasonal incidence may occur due to summer bathing, which was the circumstance of the three infections they reported.

In 1937, de Lavergne and Accoyer emphasised that many instances of "pure" meningitis were the result of bathing.

Grelland (1946) reported meningitis and conjunctivitis without jaundice or nephropathy in a boy of 10 years in Norway.

Buzzard and Wylie (1947) reported five patients who in July, August or September, 1946 had shown infection by *L. ictero-haemorrhagiae*, with meningitis and without jaundice. This is the first series of "pure" meningitis recorded in Great Britain. The patients were of ages from 9 to 23 years. The three youngest were boys who had recently been bathing in a river; the others, a cook in the A.T.S. and a young man working on a farm, had not been bathing but had worked in rat-infested premises. The cerebro-spinal

fluid in all these patients showed alterations within the range found by Troisier and Boquien (1933). All recovered completely; two suffered a relapse during the third week of the disease.

In 1947 I investigated a very interesting example of "pure" meningitis due to *L. ictero-haemorrhagiae* in one of two brothers, who both had anicteric Weil's disease.

The boy who showed meningitis was (J.R.), aged 16 years. He had frequently bathed during the summer with his brother (A.R.) in the river Stour, near Canterbury. On August 2nd he complained of headache and was admitted to hospital six days later. There were no definite neurological signs but his temperature was 101°F., and he was suspected of acute anterior poliomyelitis. The cerebro-spinal fluid on August 9th, 11th and 13th showed the following characters:-

	<u>Aug. 9</u>	<u>Aug. 11</u>	<u>Aug. 13</u>
Cells/c.cm.	13	228	60
Lymphocytes	All		All
Protein/100 c.c.	25	60	60
Chlorides/100 c.c.	680	680	710
Culture	Neg.		

On August 9th, the blood leucocyte count was 5,200 per c.mm with 79 per cent of the cells polymorphonuclear.

I examined a specimen of serum taken on August 8th and found it gave a weak reaction with *L. ictero-haemorrhagiae* at a dilution of 1 in 30 but was negative in higher dilutions and was negative with *L. canicola*. On these findings and without any clear neurological signs a diagnosis of abortive poliomyelitis was made.

A specimen of blood was taken on August 20th and it agglutinated *L. ictero-haemorrhagiae* to 1 in 1,000 and did not agglutinate *L. canicola*. Thus the diagnosis was made clear.

It may more briefly be stated that the brother, A.R., aged 15 years, was admitted to hospital on July 20th with pyrexia, abdominal pain, headache, backache, pains in the legs, vomiting and general malaise. Since the abdominal pain settled in the right iliac fossa, a provisional diagnosis of acute appendicitis was made. When he was anaesthetised for operation he had severe epistaxis and the operation was abandoned after an abdominal incision had been made.

I examined two specimens of his serum. That taken on July 21st agglutinated *L. ictero-haemorrhagiae* to a titre of 1 in 100 and was negative with *L. canicola*. The serum taken on



August 20th was positive with *L. ictero-haemorrhagiae* to 1 in 300 and negative with *L. canicola*. These patients were treated at the Kent and Canterbury Hospital at Canterbury and I am indebted to Dr. I.B. Morris, the pathologist there, for the specimens of serum and for the clinical notes and reports of cytological and chemical findings in the cerebro-spinal fluid.

Dr. J.C. Broom has told me of three other "pure" meningeal cases which he detected during the summer of 1947. With Buzzard and Wylie's cases that makes nine cases known to me in this country which might have been misdiagnosed as acute poliomyelitis during the recent epidemic of that disease. In the benign form of leptospirosis due to *L. grippo-typhosa*, *L. sejroe*, *L. mitis* (syn. *L. bataviae*) and *L. pomona*, mild meningitis is the usual form of the illness and Frey (1948) has discussed fully the points of differential diagnosis between them and anterior poliomyelitis.

In contrast to these examples of meningitis with few other manifestations, the literature of this and other countries provided many examples of Weil's disease with jaundice, in which clinical meningitis, as shown by the usual signs of meningeal irritation, was prominent.

Among 41 patients with Weil's disease, Davidson and Smith (1936) found three with severe headache combined with stiffness of the neck and Kernig's sign. In one, lumbar puncture on the 8th day of the disease showed 238 cells per c.mm (67 per cent being polymorphonuclear) and 80 mgm. of protein per 100 c.c.

Wigmore and Denning (1936) noted meningeal irritation and Kernig's sign in their patient but cerebro-spinal fluid taken on the 3rd day of the illness showed no increase in protein, globulin or cells. This is compatible with the findings at the

early stages of the twenty cases summarised by Troisier and Boquien (1933), as noted above.

In 1944, Lescher reported a patient in Derby in whom distinct meningitis preceded jaundice.

Robertson (1946) remarked on neck rigidity and Kernig's sign and stated that some of his cases in Hampshire came to hospital with a diagnosis of meningitis. From Denmark, Petersen (1944a) likewise reported meningitis with Kernig's sign and pleocytosis in the cerebro-spinal fluid.

In 1947, I saw a sewer-man who showed the disease in the form of mild jaundice and mild meningitis. A brief outline of the case is as follows:-

F.S., 33 years of age, became ill on 5th October, 1947, with pain in the back and limbs and attacks of shivering. Two days later, in hospital, he showed slight jaundice and slight conjunctivitis. The infection was a mild one and the Van den Bergh test showed 1.25 mgm. bilirubin per 100 c.c. of blood. Penicillin (50,000 units every three hours) and antileptospiral serum (Burroughs Wellcome; 40 c.c. per day) were given on the 8th, 9th and 10th October. On the 10th symptoms subsided and treatment was stopped. Pyrexia, headaches and knee pains returned next day. These ended after 5 days treatment with penicillin and returned again in a second relapse. Again treatment succeeded and a third relapse followed, which in turn finally subsided after penicillin. During these relapses, in which the severe headaches were prominent, the cerebro-spinal fluid showed 28 cells per c.mm. on the 27th day of the illness and 50 per c.mm. on the 67th day. The serum did not agglutinate *L. ictero-haemorrhagiae* at the 3rd day of the illness but did so to a titre of 1 in 1,000 on the 9th day and 1 in 3,000 on the 20th day. The cerebro-spinal fluid agglutinated *L. ictero-haemorrhagiae* to a titre of 1 in 30 on the 26th day.

jaundice

A combination of more severe/and long-lasting meningitis was described by Murgatroyd (1937). This case appears to be unique in the literature. The patient showed the usual manifestations of a moderately severe infection, but although improvement occurred at about the eleventh day of the illness there followed



a period of almost four months of incomplete recovery and irregular pyrexia. At the end of that time symptoms and signs of severe meningitis were found and the specific organism was isolated from the cerebro-spinal fluid twenty-five weeks after the onset of the disease. In the urine the leptospira were found thirty-three weeks after the illness began. The patient finally recovered after eight and a half months' illness.

Illustrating another degree of disturbance of the meninges by the infection are records of changes found in the cerebro-spinal fluid in patients showing the infection in an icteric form but without obvious meningeal irritation. For instance, Swan and McKeon (1935) found in such a patient bile-stained cerebro-spinal fluid containing 64 cells per c.mm. (all lymphocytes) and 80 mgm. protein per 100 c.c.

I saw recently a sewer-man suffering from Weil's disease with severe jaundice and anuria but no signs of meningeal irritation. Cerebro-spinal fluid was removed in order to inject nupercaine to combat the suppression of the urine. The findings in the fluid were:-

Cells 772 per c.mm. (lymphocytes 95 per cent, polymorphonuclears 5 per cent).  
 Protein 50 mgms. per 100 c.c.  
 Globulin in slight excess.  
 Chlorides 665 mgms. per 100 c.c.

It is clear, therefore, that the meninges are a common site of mild reaction in all forms of the disease and may cause obvious meningeal symptoms in a small proportion of all known cases. Mortensen (1949) has summarised reports of the involvement of the cerebral tissue and peripheral nerves, which very rarely occurs.



The connection between bathing in fresh water and meningitic forms of Weil's disease was noted by Marie and Gabriel (1936) as quoted above. This has been the experience in Holland, where Walch-Sorgdrager (1939) found that 30 out of 39 instances of meningitis leptospiroza followed swimming or sudden immersion in fresh water. Marie and Gabriel have also been confirmed by later experience in pointing to a greater incidence for this form of Weil's disease in children and adolescents, and especially in boys.

The reason why the meningeal form of the disease is specially associated with infections of boys in fresh water is the subject of discussion. In a section above it has been stated that the entry of water into the conjunctiva, the respiratory tract and perhaps the alimentary tract takes the place of entry through injured or sodden skin, in many cases of Weil's disease gained in the water by people of all ages. It has also been argued that the reasons why swimming and immersion in water cause less Weil's disease of any form in females than in males are that the former are less likely to be suddenly immersed, to bathe in dirty water or to use strokes that easily allow water to enter the mouth. There remains unexplained why among males, infections by water are more likely to occur in boys than in men. The supposition may be put forward that in both older and younger people entry of the organism by the conjunctiva and the mucous membranes of the mouth is likely to lead, by lymphatic drainage or otherwise, to rapid access to the meninges and that if the leptospira is relatively avirulent in relation to the particular person the infection may not become serious and generalised. As to the difference between men and boys, it may be suggested that in boys the tonsils and other lymphoid tissues of the pharynx may

give more ready entry to the leptospira than in a man. This line of thought appeals to Buzzard and Wylie (1947) who record with regard to it that in their series of five patients, the three boys who had been bathing showed signs or symptoms of faucial inflammation, whereas the other two patients showed no such evidence. It must be remembered, however, that sore throat is a relatively common complaint in Weil's disease in the icteric form, when the route of infection is not by the mouth or conjunctiva. Thus, Davidson and Smith (1936) found that the throat was sore and congested in just over half their cases among fish-workers, and Petersen (1944a) found it was a frequent symptom in Weil's disease in general.

### SPECIFIC TREATMENT.

#### Antileptospiral Serum.

This thesis does not consider treatment as such, but antileptospiral serum and penicillin are agents with which bacteriologists are much concerned.

Serum treatment of Weil's disease has not appeared very successful in the more recent series of instances of its use which have been collected. This is surprising, because antiserum produced in man and horses has been shown to possess protective antibodies against experimental infection with *L. ictero-haemorrhagiae*. For example, I showed that the blood of sewer-men who had not suffered from jaundice could contain both agglutinins and protective antibodies (Alston and Brown, 1935); Das Gupta (1939) compared serum from a human convalescent, serum from an immunised rabbit and serum from an immunised horse (Burroughs Wellcome) and found that under the conditions of his experiments these three sera protected guinea-pigs against *L. ictero-haemorrhagiae* for 25, 41 and 10 days respectively after they were injected into the animals; and Larson (1941) found that mice infected with *L. ictero-haemorrhagiae* could be protected by human convalescent serum or rabbit immunised serum if the serum were injected up to four days after infection, but not so late as six days.

Inada et al. (1918) considered that a considerable reduction of mortality was produced by serum treatment and very soon thereafter treatment by serum from convalescent human beings or immunised animals was used in many countries including Great Britain, where Stanley Griffith (1919) produced a serum from horses.

Walch-Sorgdrager (1939) analysed a series of 48 patients who were treated with serum and compared them with almost 100 who did not have serum. She did not compare the death rates in the two groups but found that relapses were rather more common with serum than without it and that the duration of the febrile period was the same in the two groups. From observation of a few very acute early cases she believed that serum is beneficial to such patients when it can be given during the first four or five days of illness.

Robertson (1946) reported that 12 patients were given antileptospiral serum during the first four days of the illness and all of them recovered. Of about 20 others, none of whom "received treatment until after the fourth day", 6 died. It is not clear whether these twenty patients received serum after the fourth day or not at all.

In the series of 58 sewer-men whose cases are later analysed for this thesis, I know that 13 received serum; of these 13, 2 died (15 per cent) while the case mortality rate for the other 45 was 22 per cent.

Concerning the interference of therapeutic injection of antileptospiral serum with diagnostic agglutination tests on the patient's serum, Esseveld (1937) found that in three patients not suffering from Weil's disease who were given an injection of antiserum by mistake the agglutination reaction never rose above 1 in 100 and disappeared in two of them after a fortnight. My findings in rabbits (1940) agree, in general, with this and Robertson (1946) had similar experience to Esseveld's in a patient to whom serum was given under a mistaken diagnosis. The



results of these observations are that a titre of 1 in 300 is not at all likely to be reached by injection of antiserum of the titre of 1 in 30,000, and will steadily decline.

#### Penicillin.

In April, 1944, as described in detail later, I found that eight strains of *L. ictero-haemorrhagiae* were inhibited in growth in culture by penicillin in concentration of approximately 0.11 Oxford unit per c.c. of medium. A lethal as well as a leptospirostatic effect was shown (Alston and Broom, 1944). I began a series of experiments on the effect of penicillin on infections by *L. ictero-haemorrhagiae* in guinea-pigs and found that 60 units of calcium penicillin (corresponding to a dose of 14,000 units for a man of 70 kilograms) did not protect guinea-pigs from intraperitoneal infection when the drug was given by the same route, immediately after infection and repeated 48 hours later. The animals treated with penicillin died in about the same time as the controls. I found also that penicillin had no therapeutic effect when it was exhibited first on the sixth or seventh day of infection at a time when symptoms such as increased temperature were commencing. At this time I first read the reports of Hamre et al (1943) and of Heilman and Herrel (1944) that large doses of penicillin given at frequent intervals and beginning soon after infection will protect guinea-pigs against virulent leptospira. Broom and I then collaborated on more experiments and found no effect produced by penicillin started two to four days before symptoms were expected. Finally, we tried an experiment similar to that of Heilman and Herrel and showed favourable results when penicillin in comparatively large doses was begun 18 hours after



infection and continued three times a day for thirteen days. The details of dosage in these experiments are given in the section on my own work in this thesis.

In 1944, Augustine et al. reported two experiments which showed that penicillin had no effect on survival of guinea-pigs after infection by *L. ictero-haemorrhagiae*, but that suppression of symptoms could be obtained when treatment began 38 hours after infection. Larson and Griffitts (1945) compared the therapeutic effect of 8 repeated doses of 50 - 100 units of sodium penicillin with that of a single dose of 0.5 c.c. of immune serum on young white mice inoculated with *L. ictero-haemorrhagiae*. These two treatments gave similar results, since in both cases there was survival of 95 per cent of the animals when treatment began within 48 hours and this effect decreased with time until only 28% survived when penicillin was begun in 100 unit doses at the 88th hour. Supposing that the mice weighed 50 grams each, 100 units of penicillin corresponds in body concentration with 140,000 units given to a man of 70 kilograms. Petersen et al. (1945) found that 12 strains of *L. ictero-haemorrhagiae* were susceptible in vitro to a concentration of penicillin similar to the one that I had found. They also cured guinea-pigs with 0.3 Oxford unit of penicillin given every three hours for three days, beginning four days after the animals had been infected. Chang (1946) concluded from his work that penicillin has a leptospirostatic but not a leptospirocidal action in vivo, in the conditions of growth which he used; that the therapeutic effect in guinea-pigs depends on its administration before the appearance of the jaundice or, in other words, before the liver is seriously damaged.

The general tone of these experiments is fairly consistent as to the therapeutic effect. It is that, with the doses used, penicillin is only partially successful or quite unsuccessful if it is given after the most frequent danger signal - jaundice - has appeared. It might be considered that the doses of penicillin should have been larger, but it might be remembered that experiments like those of Broom's and mine were done before the dosage of penicillin in some lesions had been raised, in successive bids, to as much as four million units a day or more for the treatment in human beings of diseases like subacute bacterial endocarditis. I feel that although the dosage of penicillin in these experimental infections might be higher and the amount given to patients in severe cases of Weil's disease be two million units a day, there is a principle of work illustrating that beyond the maximum relief that can be produced by antibacterial drugs, the patient will in general infections be left with some residuum of bacteria and his fate will depend on whether he can provide the necessary counter-action from his blood and tissues.

There have been yet very few useful reports on the use of penicillin for treatment of infections of human beings by *L. ictero-haemorrhagiae*. Bulmer (1945) collected data from the records of 16 service patients suffering from Weil's disease in Normandy. The dosage was 40,000 units given every 3 hours with a total of 1 million units. Eight of the infections were severe, 7 were moderate and one was mild; one died of uraemia and there was considered to be a greater speed of fall of temperature and fewer relapses than in untreated cases. Among 23 cases of Weil's disease in Normandy which did not receive penicillin, 2 died - one

of myocarditis and one of uraemia. The differences in this small series are not significant but the figures might be used to compile with others. Hutchison et al. (1946) found no benefit from treating with penicillin 6 of 17 British soldiers who contracted Weil's disease from bathing in the river Arno in Italy. In these patients, 15,000 units were given every three hours reaching a total of 600,000 units; the treatment began at the 6th or 7th day of illness; one of the six died and the others had serious illnesses apparently unaffected by the drug. Single cases of recovery after penicillin have been reported, for example, Hart (1944) and Carragher (1945).

#### Streptomycin.

Heilman (1945) reported that he infected hamsters with *L. ictero-haemorrhagiae* and started treatment 17 hours afterwards by streptomycin in doses of 1,000 units four times a day for 13 days. The result was that in two series all of 25 so treated survived, and all of 25 untreated died. He considered that streptomycin should be tried in human therapy, as an adjuvant to penicillin.

Wylie and Vincent (1947) recorded that cultures of 29 strains of leptospiras (including several of *L. ictero-haemorrhagiae*) were inhibited in culture by streptomycin, in concentrations which varied for different strains but were at most about 0.5 unit per c.c. Weight for weight, they found that of the preparations which they had at command, penicillin was more strongly inhibitory than streptomycin.

### SEROLOGICAL DIFFERENTIATION OF LEPTOSPIRAS.

This thesis aims at a consideration of only two of the pathogenic leptospiras - *L. ictero-haemorrhagiae* and *L. canicola* - since these are the only species which have yet been found, either free in nature or in infections of man or animals, in the British Isles. A very brief sketch will be made, however, of the species isolated elsewhere. Rather more emphasis will be given to the species which have been found in Europe since it is they that should first be sought for in the British Isles.

The relationships between species and types of leptospiras resemble the relationships of other species of bacteria, such as the salmonellas, in the overlapping of antigenic factors. There is resemblance also in the fact that a few leptospiras *L. ictero-haemorrhagiae* and less, *L. autumnalis*, *L. bataviae*, and *L. Andaman A*, are very pathogenic for man; the great majority cause only mild illness in which jaundice or meningitis, if either, occurs, is the feature which usually leads to search for leptospira in sporadic cases.

Broadly speaking, serological separation of species, types and strains has been done most thoroughly in Japan (Koshina et al., 1925), in Malaya (Fletcher, 1927), the Dutch East Indies and Holland (Schüffner, 1934; Esseveld, 1937, Walch-Sorgdrager, 1939; van Thiel, 1948) and Denmark, Petersen (1944b). Among these the fullest <sup>account of</sup> relationships, as known up to 1939, is given by Walch-Sorgdrager in the monograph referred to.

It was announced by C. Borg Petersen of Copenhagen at a congress in Amsterdam in 1938 that strains of *L. ictero-*



haemorrhagiae were divisible by agglutination tests into a complete type with antigenic factors A and B and an incomplete type containing only factor A. This was confirmed by Gispén and Schüffner (1939) who found that strains of either type could be found in rats and in human infections and that they appear to be stable. Among British strains "Jackson" was complete and "Hickey" incomplete. These writers showed that a similar relationship of complete and incomplete forms exist between *L. akiyami* A (syn. *L. autumnalis*) and *L. rachmat*. *L. ictero-haemorrhagiae* shares antigenic fractions with *L. canicola* and *L. salinem*.

*L. hebdomadis* (syn. *L. akiyami* B; related to *L. sejroe* and *L. saxkoebing*) causes mild seven-day fever in Japan.

*L. autumnalis* (syn. *L. akiyami* A, complete form of *L. rachmat*) causes leptospirosis, sometimes of severe nature, in Japan, Andaman Islands, Sumatra.

*L. andaman* A causes leptospiral jaundice with appreciable mortality in the Andaman Islands.

*L. salinem* (related to *L. ictero-haemorrhagiae* and *L. canicola*) causes mild disease in Sumatra.

*L. bataviae* (syn. Swart van Tienen strains, *L. mitis*, *L. oryzeti*) causes moderately severe disease in the East Indies and in Europe.

*L. grippo-typhosa* (syn. *L. andaman* B) causes mud-fever, slime fever or harvest fever in Europe and in the Andaman Islands.

*L. pomona* (syn. Mezzano type; related to *L. autumnalis*) produces non-fatal disease with no jaundice but frequently with meningitis in Australia and Europe.



*L. sejroe* causes mild leptospirosis in Europe.

*L. australis* A (syn. Ballico strain) causes a mild illness in Australia and Europe.

*L. australis* B (syn. Zanoni strain) has been found in Australia.

*L. poi* (~~related to *L. ballum* which has been found so far only in a mouse~~) causes mild disease in Europe.

*L. saxkoebing* (syn. Nin and Sala strains; related to *L. sejroe* and *L. hebdomadis*) causes mild leptospirosis in Europe.

*L. suis* causes mild infection in the Argentine (Savino et al., 1944).

Unnamed strains <sup>(*L. bovis*)</sup> caused leptospirosis in cattle and human beings in Palestine (Bernkopf et al., 1947).

Petersen (1944b) gives a list of leptospiras known in Europe (in addition to *L. ictero-haemorrhagiae*) with the dates of their discovery in this continent, thus:-

*L. grippo-typhosa*, 1931; Tarrasof (1931).

*L. canicola*, 1931; Klarenbeek and Schüffner (1933).

*L. sejroe*, 1937; Petersen and Christensen (1939).

*L. bataviae*, 1938 (previously known in E. Indies)  
Mino (1938).

*L. pomona*, 1938 (previously known in Australia)  
Babudieri and Bianchi (1940).

*L. australis* A, 1942 (previously known in Australia)  
Mino (1942).

*L. poi*, 1941 (Mino 1941, 1942).

*L. saxkoebing*, 1942 (Petersen 1944,b).

*L. ballum*, 1943 (Petersen 1944,c) Infection of human beings with this type has not yet been found.

Of the strains in the list above, only *L. canicola* has been found as an infecting organism in the British Isles.

Buckland and Stuart (1945) discovered by agglutination two infections by *L. grippo-typhosa* and one by *L. sejroe* in British soldiers in France. Search should be made in the British Isles for infection by these leptospiras and by *L. pomona* which causes many instances of young swineherds' disease in Switzerland (Gsell, 1946).

#### The Relationship of Saprophytic to Pathogenic Strains.

One of the most controversial subjects regarding leptospirosis is the question whether the saprophytic leptospira, *L. biflexa*, can ever become pathogenic. *L. biflexa* has been found very widely in natural fresh waters and in all manner of damp places, and it has been regarded by most workers as not capable of infecting the higher animals. Contrary views have, however, been maintained by some. Uhlenhuth and Zuelzer (1921) grew a water leptospira for a year in a medium of serum-water and found that it was then pathogenic for guinea-pigs. Uhlenhuth and Hermann (1927) "blocked" the reticulo-endothelial system of young wild rats, inoculated them with the water strain and afterwards isolated pathogenic leptospira from the animals. Baermann and Zuelzer (1928) also were of the opinion that on prolonged cultivation water strains became pathogenic to human beings. Later, Zuelzer (1936b) stated that, as judged by agglutination and lysis, there are certain gradual transitions from the pathogenic strains to the free-living leptospiras and that *L. grippo-typhosa*, which is the cause of swamp-fever, is related to the other pathogenic strains. On the contrary, many workers have not been able to confirm these findings, and the majority of observers (such as van Thiel, 1933) do not consider that the saprophytic water strains can ever become pathogenic. Taylor and Goyle (1931) suggested that there is

always the possibility that Uhlenhuth and his colleagues were working with water strains obtained from sources in which pathogenic strains might also have been present, and Brown and Davis (1927) found that strains of *L. ictero-haemorrhagiae* and of *L. biflexa* were serologically distinct. In addition, during the procedure of isolation and maintenance of virulent strains from patients suffering from Weil's disease, it is common experience (Alston and Brown, 1937) to demonstrate that cultures of pathogenic leptospira lose their pathogenicity for guinea-pigs after subculture for about four months. Therefore, if the pathogenic strains lose virulence in this way it is difficult to see how the non-pathogenic strains acquire it in the course of the same procedure.

tion of guinea-pigs with the organism. I have also taken part in the prevention of the infection in water-rats, and in the serial subcultures of it by maintaining in co-cultivation plates and by giving evidence to the Departmental Committee of the Home Office on Fishermen's Compensation for Industrial Diseases.

A short account of the history of London may appropriately be given. This drainage system (Burgoyne, 1930) covers 150 square miles, on both sides of the Thames, on which the local population was 5½ millions before the Second World War. The territory includes all the County of London and some other districts of which the natural drainage is through the County area towards the Thames. Construction of the present system of drains was commenced about a hundred years ago, on a plan prepared by Mr Joseph Bazalgette, Chief Engineer of the Metropolitan Board of Works.

MY OWN EXPERIENCE OF LEPTOSPIROSIS ICTERO-HAEMORRHAGIAE.

My own experience of Weil's disease first centred round infection of sewer-workers in London and later extended to other circumstances by which the disease is spread. In addition, I have detected the presence of the organism in rats and mud in sewers and tested the serum of rats for antibodies to *L. ictero-haemorrhagiae*. I have detected antileptospiral antibodies in the serum of sewer-men who had not suffered from jaundice, demonstrated the rapidity with which antibodies injected into rabbits disappear from the animals' circulation, demonstrated the sensitivity of several species of leptospira (including *L. ictero-haemorrhagiae*) to penicillin and (jointly with J.C. Broom) shown that penicillin has an effect on the result of experimental infection of guinea-pigs with the organism. I have also taken part in the prevention of the infection in sewer-men, and in the social consequences of it by assisting in compensation claims and by giving evidence to the Departmental Committee of the Home Office on Workmens' Compensation for Industrial Diseases.

A short account of the sewers of London may appropriately be given. This drainage system (Humphreys, 1930) covers 150 square miles, on both sides of the Thames, on which the human population was  $5\frac{1}{2}$  millions before the Second World War. The territory includes all the County of London and some other districts of which the natural drainage is through the County area towards the Thames. Construction of the present system of drainage was commenced almost a hundred years ago, on a plan prepared by Sir Joseph Bazalgette, Chief Engineer of the Metropolitan Board of Works.



The removal of refuse of all kinds from London had been for centuries a difficulty which it was beyond the engineering or administrative powers of the community to solve adequately. At the beginning of the nineteenth century the invention of the water-closet offered facilities, before unknown, for the entire removal of sewage. At first the water-closets were made to discharge into cesspools, the ancient receptacles for offensive household refuse, and the contents of the cesspools were removed from time to time. The large addition thus caused to the contents of the cesspools made it necessary to introduce overflow drains running from them into the street sewers. The discharge of offensive matter into the sewers was a penal offence up to about 1815; it then became permissive to drain houses into sewers and, in 1847, it was made compulsory.

The sewers were originally banked up water-courses, intended solely for carrying off the surface drainage and included such streams as the Westbourne, the Fleet river, Walbrook and Shoreditch. Before the nineteenth century, many of these had been culverted and placed underground. In 1847, control of sewers was given to the Metropolitan Commission of Sewers, who in a few years practically abolished cesspools - about 200,000 in number, made water-closets compulsory and drained them into the sewers. Since the sewers drained into the Thames, the river became alarmingly polluted.

To overcome this pollution, the present system was constructed by the Metropolitan Board of Works, commencing activities in 1855. The solution of the problem which was adopted was to build a series of new sewers, running from West to East, called intercepting and outfall sewers which, under normal circumstances,

carry all the drainage from the older system of sewers to outlets into the Thames at Beckton on the North bank of the river, and Crossness on the South Bank. At these points, sewage is precipitated and concentrated to some degree so that some of the supernatant, relatively clean water is allowed to flow into the Thames and the sludge is taken in special boats to be discharged in the North Sea at the mouth of the river.

The outlets of the original sewers into the Thames within the County of London are allowed to discharge into the river only for relief of storm rainfall, although special storm relief sewers are the principal means of meeting this emergency.

The completion of this plan and the working of it have been in the hands of the London County Council since 1889. The sewers described make up the system of "main drainage" and they are fed by sewers constructed in the streets and fed in turn by drains from the buildings. The street and local sewers are in the care of the separate Metropolitan Boroughs and the drains are the responsibility of property owners. The London County Council own 404 miles of sewers in their main drainage system and the local sewers in charge of the Borough Councils are estimated at 2,500 miles.

Work in the sewers is mainly of two kinds. About 800 men are employed by the County Council or by Borough Councils in cleaning and maintenance and, in addition, before the Second World War about 200 were engaged in building by public works contractors. The men employed in cleaning are known as "flushers" and both they and the "builders", in general, continue in sewer work for many years. The flushers' work largely consists of removing deposits of solid matter from the walls of the sewers and from the bottom

of the stream at places where mud settles. They scoop this mud into containers, wheel it to the man-holes and raise it to the surface for removal. Builders are chiefly occupied in re-building the floors and walls of worn sewers and, in doing so, handle a great deal of broken brick work covered with mud (photographs I and II). This seemed to be the greater risk in the building work, which causes a higher rate of Weil's disease among the builders than the flushers.

Rat infestation of the sewers is general, but not so unavoidable as might be thought. It is greater in some parts of London than others and could be greatly reduced if drains from buildings were kept in better repair. The rats do not live to any considerable extent in drains or sewers, but in the buildings or ground drained by them and they enter the drains by breaches in their continuity. As a rule, few rats are seen during working hours, but they come into the sewers by night leaving foot-prints on the mud (photograph III) and faeces which often becomes covered with fungus (photograph IV). There has been legislative power for a long time to compel property owners to keep their drains in good repair but it is only quite recently that the Government and local authorities have combined to make the drains intact in some of the worst affected parts of London. Since 1941, poisoning of rats in the London County Council sewers has also reduced their numbers. This is done regularly twice a year throughout the system.

Other ways in which the risk of infection is reduced are mentioned later in connection with hygiene.

Experience in Diagnosis of 79 Infections.  
The suggestion that sewer-workers might be specially

liable to Weil's disease was made to me in 1933 by Dr. J.A.H. Brincker, a senior medical officer in the Public Health Department of the London County Council. He had suspected that some of the instances of death with jaundice among sewer-men might be of this nature, although the records of illness and post-mortem examination appeared confident in diagnosing the infections otherwise. In reading ten such records it appeared that pneumonia or pulmonary congestion with hepatitis were the features on which attention was centred, and the haemorrhages that were noticed were attributed to the jaundiced state. It seemed likely that some of the patients had suffered from Weil's disease.

From the beginning of 1934, therefore, I was on the outlook, with the assistance of the Chief Engineer's Department, London County Council, for jaundice among sewer-men. As a preliminary part of the work, the presence of living *L. ictero-haemorrhagiae* was sought in the sewers and especially in the sewer slime with which the workmen inevitably come into contact. No effort was made to estimate the proportion of large numbers of rats caught in sewers from which leptospiras could be isolated, because the fact of a varying but often high incidence of infection of rats by the leptospira was known from surveys in this and other countries (see Table 2.); but by inoculation of guinea-pigs with kidney and liver tissues from rats caught in the sewers in eight different parts of London, five strains of virulent *L. ictero-haemorrhagiae* were obtained at that time. The rats were caught during the night in traps laid in the sewers and were brought alive (one to four from each area) to the laboratory and killed by chloroform. The liver and kidneys of each rat were cut up and considerable proportions of them ground with sterile sand



and saline to make a suspension for injection. The suspensions from all the animals from each area were mixed and two guinea-pigs were inoculated with each mixture. Four of the strains were obtained by subcutaneous inoculation of such pooled tissues, and the leptospira were isolated in Noguchi-Wenyon medium from the heart blood of guinea-pigs which had died or were killed after jaundice had developed. On one occasion, when intraperitoneal inoculation was used the circumstances of the isolation may be mentioned. In this instance it was thought that a few inactive leptospira were present in a preparation from the rat's kidney examined by dark-ground illumination. A guinea-pig was inoculated intraperitoneally with a suspension of ground kidney tissue and remained alive and apparently well for a period of six weeks and was then killed. No evidence of jaundice, haemorrhage, or other lesion suggesting leptospiral infection was found at autopsy, and no leptospiras could be seen in preparations of blood, liver or kidney examined by dark-ground illumination. Another guinea-pig was inoculated subcutaneously with the tissue of the first and it died thirteen days later showing jaundice of the subcutaneous tissues and haemorrhages in the thoracic and abdominal organs - a typical picture of leptospiral infection. The demonstration of leptospira in the blood and tissues by dark-ground illumination was doubtful, but a pure culture of these organisms was obtained from the blood by inoculation of Noguchi-Wenyon medium.

By a similar technique of inoculating guinea-pigs by subcutaneous injection or by scarification, pathogenic leptospira were demonstrated in a specimen of slime from the floor of a sewer near the spot where Dr. Fairley's patient had been working immediately before his fatal infection and, in another specimen,

from the outlet of a house-drain into a different sewer. It is worthy of note that in the case of each of these specimens, only one out of two guinea-pigs inoculated in the same way and at the same time succumbed or showed any evidence of infection. Twenty-eight similar specimens of slime from the floor or lower part of the walls of the sewers or from the outlets of drains produced no sign of infection in the animals inoculated with them, and six specimens of the small, slimy stalactites which form on the roof and contain chiefly gritty material from the cement of the brick-work did not give rise to any evidence of infection in guinea-pigs. In the inoculation experiments described, young guinea-pigs of about 300 g. weight were used, but as has just been noted they were clearly unequal in susceptibility to infection by the same route, even although temperature records were not taken for detecting slight, non-fatal reactions. In isolating the leptospira from both slime and rat tissues in which the leptospira were probably very scanty, inoculation by subcutaneous injection or by rubbing inoculum into scarified skin surface was found more effective than intraperitoneal injection with guinea-pigs of the size used (300 g.), which was the smallest available.

In addition to isolating the leptospira from tissues, the serum of ten rats was examined for antibodies by the adhesion test (Brown and Davis, 1927), which will be described later. Eight of the ten sera gave positive adhesion tests in dilutions of 1 in 4 to 1 in 64 and two gave no reaction.

In 1938, for evidence in a court action for compensation brought by a sewer worker, 11 rats caught in a sewer in Islington were examined by guinea-pig inoculation and *L. icterohaemorrhagiae* thus demonstrated in five of them. The same

organism was similarly demonstrated in two specimens of slime from the same sewer as the rats.

The results recorded so far may be summarised:-

Examination of rats: 5 strains of *L. ictero-haemorrhagiae* from eight groups of rats, each group from one area of sewers (1934).  
5 strains of *L. ictero-haemorrhagiae* from 11 individual rats in one area (1938).  
8 rats out of ten from different parts of London showed antibodies by adhesion test.

Examination of slime: 4 specimens of slime out of 32 from floor or lower wall of sewers, gave evidence of *L. ictero-haemorrhagiae* by guinea-pig inoculation.

Examination of "stalactites":  
None of the six specimens gave evidence of *L. ictero-haemorrhagiae*.

Slime for examinations was collected from the sewers in the mornings when infection of it by the rats was recent, it was brought to the laboratory, <sup>and</sup> inoculated into animals as quickly as possible. On one occasion ~~when~~ leptospira were found virulent in sewer mud 24 hours after delivery to the laboratory when, on December 22, 1936, three samples of mud were not used at once, but each was diluted by adding to it  $\frac{1}{3}$  of its volume of sterile water and it was placed in the cold-room overnight. Twenty-four hours later, two guinea-pigs were inoculated from each specimen by rubbing some of the mud on scarified and shaved abdominal skin. Both of the animals connected with one specimen of mud died 10 days later with typical lesions and the leptospira were seen by dark-ground illumination. In contrast to this, a specimen of sewer mud was infected with a virulent culture of *L. ictero-haemorrhagiae* on January 20, 1937, was thoroughly mixed and proved virulent to two guinea-pigs inoculated with it. After half of it had been left

at room temperature overnight it was not virulent to either of two guinea-pigs inoculated in the same way as on the previous day. The other half of the specimen was divided into two equal parts and each was exposed, immediately after the mud was infected, to an ultra-violet lamp under the same conditions of distance (18 inches) from light, depth of specimen and area of surface. The portion exposed in this way for 5 minutes was virulent to two guinea-pigs inoculated, and the portion exposed for 10 minutes produced no reaction in either of two guinea-pigs.

In July, 1934, I had my first opportunity to take part in diagnosing leptospiral jaundice in a sewer-man (S.B.) by demonstrating leptospira and culturing a pure strain of *L. ictero-haemorrhagiae* in Noguchi-Wenyon medium from a guinea-pig inoculated with urine taken at the 13th day of the disease. This patient's illness began on June 26, 1934. Six weeks before, on May 12, the illness began of (C.J.) whose case, as the first proved example of a sewer-man infected with Weil's disease in Great Britain, was published by N.H. Fairley on July 7, 1934.

In November, 1933, a sewer-worker (F.W.) was treated in the London Hospital for haemorrhagic jaundice, considered clinically to be Weil's disease. Blood serum was taken at the time and was found a year later by Professor S.P. Bedson to give a positive reaction in the adhesion test in dilutions up to 1 in 400. I have included this patient in the list of Weil's disease in London sewer-men.

In July, 1934, I confirmed the clinical diagnosis of Weil's disease by adhesion test in another sewer-man (H.S), and in November, 1934, by the same test in a third (E.J.). The



three cases in which I had done the essential bacteriology in 1934 were published together (Alston, 1935). Including these three I have made, up to July, 1948, all or part of the bacteriological and serological examinations which established diagnosis of Weil's disease in 40 London sewer-men. These, with 16 similarly diagnosed by Major H.C. Brown or Dr. J.C. Broom, and two by Professor S.P. Bedson, (making a total of 58) are shown in Tables 8 & 9.

The methods which I used in different cases were culture or guinea-pig inoculation of blood during the first week, a serological test after the fourth day of illness and culture and guinea-pig inoculation of urine at any time after the first week. The details of the methods are as follows:-

See Alston, "The Laboratory Diagnosis of Leptospiral Infections" in "Recent Advances in Clinical Pathology", Churchill, London, 1947, p.115.

#### 1. Blood.

For culture or inoculation, blood is allowed to clot (unless it can be added to culture medium at the bedside); anti-coagulants have not been used.

- (a) Culture has been made by adding about 1 part of fluid blood or of serum and broken clot to 9 parts of medium. Noguchi-Wenyon, Fletcher's, Schüffner's have been used at different times. The formulae of these are:-

##### Noguchi-Wenyon medium.

Mix together rabbit's serum 1.5 parts, <sup>in</sup>Rüger's solution 4.5 parts, 2 per cent agar medium 1 part. Ensure complete mixing of the constituents and tube in 3 to 5 c.c. amounts. (Liquid paraffin is not needed in this modification).

##### Fletcher's medium (broth).

For each tube of medium add 0.5 c.c. of Lemco broth (pH 7.4) to 3.0 c.c. of glass distilled water and sterilise in the autoclave; add 0.25 c.c. of rabbit serum recently inactivated by heating at 56°C., for half an hour and passed through a Seitz filter. Incubate the tubes at 37°C., for 24 hours to test their sterility. Incubation for growth of leptospira should be at 30°C. Growth is maximum usually 3 - 7 days.

Schüffner's Medium.

Add together tap water, 1,500 c.c.; Witte's peptone 1.5 gm; Ringer's solution 300 c.c. and Sorensen's solution (pH 7.2) to make a final pH of between 6.8 and 7.2. <sup>Tube</sup> The medium in amount of 3 c.c. and 0.3 c.c. of fresh rabbit serum, added to each tube. The medium is then heated at 56°C. for half an hour and incubated (to prove sterility) overnight. Some specimens of rabbit serum are better than others and a small degree of haemolysis of the rabbit blood seems helpful. This medium gives a growth of *L. ictero-haemorrhagiae* in 3 - 6 days when incubated at 30°C.

- (b) Inoculation of blood into guinea-pigs. This has been done with fluid blood or serum mixed with triturated clot; some of the inoculum has been rubbed on to freshly shaved and scarified abdominal skin of young guinea-pigs and, in addition, some has usually been injected subcutaneously and intra-peritoneally. By this combination, up to 3 c.c. of patient's blood can be inoculated into each guinea-pig.

- (c) Serological Adhesion Test (Brown & Davis, 1927.).

The following reagents are required: (1) the patient's serum; (2) a young broth culture of *L. ictero-haemorrhagiae*; (3) a saline suspension of a young culture of *B. coli* or other similar organism; (4) a fivefold dilution in saline of fresh guineapig's serum (this is not required if the patient's serum is tested on the same day as the sample is taken).

One volume (about 20 c.mm.) of each of the above reagents is placed in a small agglutination tube and the contents mixed; a control tube contains known normal serum in place of the patient's serum. The tubes are incubated at 37°C. for thirty minutes, and then a small drop is placed on a microscope slide, covered with a cover-slip and examined by dark-field illumination. In the event of a negative reaction the leptospira will be seen swimming freely and totally unimpeded by the presence of bacteria. In a positive reaction the bacteria will be seen to be firmly adherent to the leptospira.

- (d) Serological Agglutination Test.

I have used the Schüffner method as described by Davidson et al.

(1934). It is unfortunate that stable suspensions of leptospira for agglutination tests cannot be prepared with certainty and careful controls of the state of the antigen as to its response to serum known to contain agglutinins and its insensitiveness to negative serum must be made. It may happen that the culture of a particular strain of *L. ictero-haemorrhagiae* (often one which has lost its virulence for guinea-pigs) will supply a satisfactory antigen for a long

time, but suddenly lose its agglutinability or become clumped in culture, in saline or in known negative serum. I have had experience, also, of false positive results with serum of patients suffering from jaundice not due to Weil's disease. Broom and Brown (1943) report similar findings with a Jackson antigen which had previously been specific and was so when antigen was made from later subcultures of the strain. Such non-specific antigens may be negative with serum from anicteric patients. It is for these reasons that these serological tests have not yet become usual practice in most bacteriological laboratories.

For making the antigen, cultures in Fletcher's broth have been selected by making sure that they do not show clumping of the leptospira when examined microscopically and they are killed by adding to 10 c.c. of culture 0.2 c.c. of the following mixture (Burke, 1933): Formalin (commercial) 50 c.c., pyridine 10 c.c., water 150 c.c.

The Jackson strain of *L. ictero-haemorrhagiae* isolated from Fairley's case of a London sewer-man has been used during most of my work.

It has been my practice, following Schüffner, to dilute the patient's serum with peptone water in small amounts in the cups of an artist's palette, obtaining dilutions of 1 in 5, 1 in 15, 1 in 50, 1 in 150, up to 1 in 15,000; when an equal amount of antigen has been added, final dilutions of 1 in 10, to 1 in 30,000 are produced. Control tests with known negative and known positive serum are added. They are all left overnight at room temperature and read by placing a drop of each between a slide and a cover-slip and examining them with the dark-ground microscope with  $2/3"$ ,  $1/6"$  or  $1/12"$  lens.

I have also used the rapid method of agglutination test described by Brown (1939). For this the formalised culture of Fletcher's medium is concentrated by adding to it one gram of saponin per litre and centrifuging at about 6,000 revolutions per minute for half an hour. The mixtures of serum and antigen are made as before in a palette and the whole of each mixture is transferred to a section on the surface of a long, thick glass slide. The sections are separated from one another by shallow troughs and the slide is mounted on a rocker. After rocking for ten minutes a result is obtained which is equal in titre to that of the overnight test with unconcentrated antigen.

Broom (1948) has given an excellent review of the serological reactions to be found in patients with Weil's disease. He concludes that the agglutination reaction with antigen of *L. ictero-haemorrhagiae* even in dilutions no higher than 1 in 10 is very specific for a leptospiral infection of either present or past existence; and that doubt of its significance of infection at the time the blood was taken is due to administration of immune serum or the presence of residual antibodies from past infection. He found that in the first of these possibilities the titre decreased rapidly, as I found in rabbits, (Alston, 1940) and in the other it is stationary. In practice, agreeing with my own experience, he did not find titres higher than 1 in 300 from subclinical infections in the past and therefore he believed that a single finding at 1 in 1,000 may be taken as confirming clinical suspicion of Weil's disease; a rising titre of agglutination is better evidence still and often necessary when the first result is below 1 in 1,000. As regards the theoretical risk of titre of 1 in 1,000 or higher being due to <sup>previous</sup> infection, in the early stages of Weil's disease it is very unlikely that a new infection would commence with antibodies in the serum of that concentration; I do not know of a proved example of a second attack of Weil's disease, excluding relapses. Broom points out the risk of confusion between agglutinins caused by *L. ictero-haemorrhagiae* and by *L. canicola* and gives records of a patient suffering from the former infection who at the 16th day of the disease showed greater agglutination of *L. canicola*, but at the 26th day the reaction to *L. ictero-haemorrhagiae* was much stronger. This phenomenon occurs more with strains of *L. ictero-haemorrhagiae* of the "incomplete" type and since infections by *L. canicola*



are being more frequently reported in Great Britain than formerly (see section on *L. canicola*, in this thesis) this will need more and more to be observed.

## 2. Urine.

The patient's urine has been neutralised in vivo by giving him alkalis or immediately after it has been passed, to lessen the destructive action of acid on the organisms. I have not attempted to centrifugalise the urine since this is an uncertain method when few leptospiras are present but, as with blood, I have inoculated 3 c.c. of urine by rubbing it on scarified skin and by subcutaneous and intraperitoneal injection.

In addition to the 40 London sewer-men whose infections I have examined bacteriologically as recorded in Table 8, 18 others were investigated to my knowledge by H.C. Brown, J.C. Broom or S.P. Bedson and these are recorded in Table 9. For convenience these 58 cases have been analysed together for this thesis.

The incidence of the infections by month has already been commented on in the section on seasonal incidence of the disease and the concentration of cases in summer and autumn months (especially September, October and November) is shown in Table 5.

Where age is known, in forty-six instances, the range is from 25 to 67 years. The distribution periods of 10 years is given in Table 10 and shows the largest numbers of cases in the fourth and fifth decades of life.

The length of time that patients had been working in sewers before infection is known in 19 instances. The range is from 3 weeks to 20 years. In 8, the period was less than one year and in 2, more than 10 years. It is clear, therefore, that the risk is greatest at the beginning of the employment.

When there are considered, the twenty-nine patients before the Second World War, of whom it is known whether they were

flusher or builder, 15 of the former and 14 of the latter are found. The number of flushers at risk is four times the number of builders so that the greater risk of infection for builders is confirmed although not to the higher degree already referred to. After the Second World War began, building work very greatly decreased and has remained at a minimum since it ended. Since September, 1939, only 2 infections are known in builders and 12 in flushers.

The outcome of the illness is known in 56 instances; in 12 of these (21 per cent) the patients died.

The experience of the periods of the illness at which blood or urine contained leptospira capable of infecting guinea-pigs was in line with established expectations. During most of the years of the Second World War cultures and guinea-pig inoculations were not made, but attempt was made in 19 cases to obtain the infecting organism from blood or urine or both. *L. ictero-haemorrhagiae* was obtained from the blood of 8 patients; in 6 the time of illness was known and was from the fourth to the seventh day; in nine instances when blood was taken from the 24th day, animal inoculation failed. Urine of the 13, 16th or 9th to the 23rd day conveyed infection to guinea-pigs from three patients and three specimens taken earlier in the illness (6th to 9th days) and five taken later (17th to 40th day), failed.

With regard to leucocyte counts these are recorded for fifteen patients. In thirteen, the total count is 9,000 per c.mm. or above, the highest figure being 25,000. In two patients at the end of the first week of illness the count was 4,800 in one and 6,600 for the other. Robertson (1946) has noted that counts within normal limits may be found in the early part of the illness.

Reduction of the red corpuscle count and of haemoglobin concentration is usual. The former often falls to 2.5 million per c.mm. and the latter to 50 per cent. In eleven sewer-men the lowest recorded counts during the acute stage of the illness were in millions per c.mm. - 2.4, 2.4, 2.7, 2.7, 2.8, 3.0, 3.6, 3.9, 4.7, 5.0, 5.0. Recovery to normal levels is usual during convalescence.

Among the 40 sewer-men whom I investigated three were anicteric (8 per cent). This is fewer than might be expected when every chance is given to the doctors of sewer-men to have any form of illness tested for Weil's disease (as described later), but the few mild cases distinguished may reflect the strong-minded attitude of patients and doctors not to seek obscure causes for trivial illnesses. Isolated anicteric infections are noticed in this country from time to time. Stewart and Witts (1944) collected four such in a child of four years who fell into a ditch, a doctor who lived near a rat infested stream, a boy bathing in the same stream (the Cherwell) and an air-man scratched by a rat; all recovered. I have just investigated an unusual case (August 1948, unpublished). This is of a man 26 years who took a cycling holiday from Dieppe to the Rhône valley, and bathed and drank water in rivers and streams as a matter of principle, as it were, to get a full taste of the country he was visiting. On return to England he became ill with a pyrexial illness, abdominal pains and a rash on the abdomen. He had distinct headache, but not any more definite evidence of meningeal irritation. Typhus fever was suspected and a Weil-Felix test was requested. Through a confusion of the discovery of Adolph Weil with that of his name-

sake of thirty years later, the patient's blood was sent to me. As a result I carried out a leptospiral agglutination test as well as the Weil Felix reaction. The latter was negative but to my surprise the serum agglutinated *L. ictero-haemorrhagiae* to 1 in 3,000 and *L. canicola* to 1 in 100. These results were confirmed by Dr. J.C. Broom. The patient has not shown jaundice, is no doubt an example of mild meningitis and abdominal pain such as was shown by the boy A.R., mentioned in the section on meningitis.

Table 7, recording highest detected concentrations of urea in the blood in Weil's disease, has been commented on already. Many of the figures were taken from London sewer-men.

My Investigations in Weil's Disease, excluding Sewer-men.

In addition to the forty sewer-men for whom I carried out diagnostic bacteriology and serology, I detected Weil's disease in thirty-nine other persons between August, 1935 and July, 1948. The statistics available for these are less complete than for the sewer-men because many of the patients were in parts of the country distant from London or on service in the British or U.S. forces, but some information can be summarised as follows:-

1. The occupation is known in 32 instances, viz.
 

British Army or U.S. Army	12
Bathing	7
Fishmonger	2
Sudden immersion, bargeman, bricklayer, camper, worker in greyhound stadium, plasterer, railway guard, soldier at bomb-disposal work, stone cobbler, waiter, woman (whose husband was infected at the same time, in circumstances unknown) - one each.	

2. I know the outcome in twenty-two patients, of whom five (23 per cent) died.

Among the 79 cases, of sewer-men and others, which I have investigated, there are 31 in whom more than one specimen of



blood was examined for agglutinins. Among these, there were seven instances when a negative test in the early days of the illness was followed by a positive one later; five of these negative results were in serum taken at the 2nd, 2nd, 4th, 6th and 7th days. A reaction at dilutions of 1 in 10 was found once on the fourth day and, in another case, one of 1 in 30 at the sixth day. (Table 15).

Agglutinins & Protective Antibodies in Blood of Sewer-men.

In 1935, I examined with Major H.C. Brown, the serum of certain sewer-men in order to look for agglutinins and antibodies which could protect guinea-pigs against infection by virulent *L. ictero-haemorrhagiae*. Blood was obtained from one sewer worker who had suffered from jaundice and from forty-five who declared that they had never had that symptom in connection with any illness since they began sewer work. The antigen which I used in the agglutination test was the Jackson strain of *L. ictero-haemorrhagiae* isolated in 1934 and Major H.C. Brown confirmed some of the tests by this method and re-tested some by the adhesion test. Similar results were given by the two methods. The man who gave a history of jaundice stated that the illness occurred twenty-three years ago, eighteen months after he had begun sewer work, and the end titre of his serum in the agglutination test was 1 in 300. Among the 45 who had no recollection of jaundice, nine (20 per cent) showed by one test or, when both methods were applied, by each of them, a reaction with an end titre of 1 in 30 in five instances and 1 in 100 in four instances. The period of sewer work of the nine reactors varied from eight to thirty-seven years, and those who showed no reaction had a similar range of service.

As a control to these results sera submitted for Wassermann reaction from 107 men, women and children were examined, and none of these gave a reaction when tested by the agglutination method in a dilution of 1 in 20.

Next, I endeavoured to determine whether the presence of agglutinins is necessarily an indication of protective antibodies in the serum of sewer-men and I showed that four sera which were positive in the agglutination test had the power of protecting guinea-pigs from the effect of injection of virulent *L. ictero-haemorrhagiae*; the sera of two adult men chosen from the general population and showing no agglutinins were used as controls in this experiment. The experiment was carried out with twelve pairs of young guinea-pigs of the same breed and matched by weight as between the pairs as well as possible. Each pair of animals were given intraperitoneal injections of 0.5 or 1.5 c.m of one of the six sera used, and after an interval of one hour, each animal was injected intraperitoneally with 0.2 c.cm of a seven days old culture, diluted in physiological saline, of a virulent strain (Mc.Vady) of *L. ictero-haemorrhagiae* in a rabbit serum broth medium. The diluted culture contained one or two leptospiras in each dark-ground field when examined with a 1/12" objective and 10x eye-piece. The result of the experiment was that seven out of the eight of the guinea-pigs treated with one or other of the control sera died within six to seven days after infection - all seven showed typical lesions of haemorrhage and jaundice, and in one of each pair of animals leptospira were looked for by dark-ground illumination and were demonstrated. The single survivor among the eight control animals was one of the

pair treated with 1.5 c.cm of one of the sera. In contrast, only two of the animals treated with the sewer-men's serum died, and the remainder survived and were perfectly healthy five weeks after the time of infection. The two animals which succumbed died at nine days and fifteen days after the commencement of the experiments, in the second case the death being markedly delayed compared with the deaths of the control animals. The guinea-pig which died after nine days had received 0.5 c.cm. of one of the specimens of serum and that which died after fifteen days received 1.5 c.cm of another serum. The protocol of the experiment is given in Table 11.

It is considered that this experiment demonstrated that protective antibodies accompany agglutinins in the serum of certain sewer-men.

This was published by Alston and Brown (1935); the remainder of the work reported in that article was done entirely by Major Brown. The protective experiments were done entirely by me at Archway Hospital and the agglutinin tests were done by me there and repeated or re-tested by the adhesion test by Major Brown at the Wellcome Institute.

#### Effect of Therapeutic Serum on Agglutination Tests.

In 1940, I made some experiments to know how quickly the agglutinins of antileptospiral serum disappear after they have been injected into rabbits. This was done to throw some light on the value of the agglutination tests in human beings after the injection of therapeutic serum in Weil's disease.

The answer to this depends on knowing how quickly the injected antibodies decrease in the blood stream, and the matter could best be settled by injecting antileptospiral serum into

healthy, human volunteers and performing agglutination tests on serum taken from them at intervals after the injection. It was not possible to do this; but the rate of disappearance of agglutinins was watched in four rabbits, when it was found that the antibodies decreased to a very low titre within 48 hours in three and within 120 hours in the other. The rabbits were young adults of approximately equal weight, and were kept under the same conditions. Dr. W.W. Kay calculated the volume of the plasma of one of the animals by the injection of Congo red and the determination of the dilution of it in the plasma by the Pulfricht photometer. The volume of it was found to be 67 c.cm., and this was assumed for the other three animals. On this basis an intravenous injection of 0.67 c.cm of antileptospiral serum (Burroughs Wellcome) was made into each animal after a preliminary specimen of blood had been withdrawn. Small amounts of blood (1 to 2 c.cm.) were taken from an ear vein at periods of from five minutes to five days afterwards. It was found that before the animals were injected their serum did not agglutinate the leptospira significantly.

By injecting 0.67 c.cm. of the therapeutic serum into 67 c.cm of plasma of each rabbit a hundredfold dilution was made; and therefore, since the titre of the therapeutic serum was 1 in 30,000, it would not be expected that the rabbit's serum, taken after the injection, would agglutinate the leptospira in a dilution of more than 1 in 300. Actually this power of agglutination was not reached at all in one rabbit, and was found weakly in two after the first five minutes only and in one at seven hours, twenty-four hours and forty-eight hours.



Table 12 shows that the decrease of agglutinins is variable, but in three of the animals the reduction at forty-eight hours was to a concentration of one-tenth or less of that calculated from the dilution of the therapeutic serum in the animals' plasma.

Assuming a similarity in this respect between the rabbit and the human being, when serum has been given to a patient the injected antibodies decrease rapidly. This may be applied to clinical medicine. In order to estimate the volume of the patient's plasma the chief observation needed is the body weight. The proportion of plasma in the total blood volume can be found by the haematocrit or, if red blood corpuscles and haemoglobin are normal in amount, it may be regarded as 55 per cent. Then, taking the specific gravity of plasma as 1 and the weight of blood as one-fifteenth part of the body weight, the plasma volume can be calculated in cubic centimetres. Thus a patient of 12 stone with plasma volume equal to 55 per cent blood volume would be calculated to have 2,750 c.cm of plasma, and an injection of 40 c.cm. of serum would be diluted approximately seventy times. The highest agglutinative titre produced in the patient's plasma (or serum) can be calculated from the original titre of the therapeutic serum and the dilution produced in the patient's plasma. The result of tests on serum taken at any time after the therapeutic injection can be compared with this calculated result; and if the observed titre is higher than the calculated one the result will appear more and more significant, according to the extent of the difference; while in the serum taken forty-eight hours or more after the therapeutic injection, a titre equal to the calculated one will be possibly significant.

It appeared, therefore, that after an interval of seventy-two hours the effect of a therapeutic injection of anti-leptospiral serum on an agglutination test of the patient's serum could be ignored. If the interval between the injection and the test were less than seventy-two hours the test would still be useful if the effect of the antibodies injected is considered in the way indicated.

These results were published (Alston, 1940) and borne out by experience in human beings (Robertson, 1946).

Action of Penicillin on Leptospira and on  
Leptospiral Infections in Guinea-Pigs.

In 1944, I tested at Archway Hospital the action of penicillin on the growth of nine strains of *L. ictero-haemorrhagiae*. At Archway Hospital also, I made two experiments on the treatment by penicillin of guinea-pigs infected with *L. ictero-haemorrhagiae*. These experiments, as explained below, showed no effect of the drug. Subsequently I combined with Dr. J.C. Broom in two other experiments at the Wellcome Institute and we shared the chief burden of the work in the form of injections of guinea-pigs at 10 p.m. and, in one experiment, in the early morning as well. (Alston and Broom, 1944).

I did the in vitro experiments first, in April, 1944. Nine strains of *L. ictero-haemorrhagiae* were used. Six had been obtained from human infections (Jackson, Hickey, Wijnberg, K.L., Barber, Rachmat); two had been isolated from rats (wild rat and W.P.R.L.); and one from a dog (L.56). Some of these strains are virulent for guinea-pigs and others are completely non-virulent. For the test, a solution of penicillin was prepared containing 200 Oxford units per ml., and serial fivefold dilutions were made

from it. 0.25 ml. volumes of these dilutions were added to 3 ml. of Fletcher's broth. After mixing, the medium was seeded with 0.25 ml. of a heavy culture of leptospira. The final concentrations of penicillin were thus 50, 10, 2, etc., down to 0.016 Oxford unit in 3.5 ml.

In some later experiments the two highest concentrations were omitted. Most of the tests were carried out with calcium penicillin; in a few parallel experiments crude filtrate of the growth of *Penicillium Notatum* in a protein-free medium (Alston, 1944) was used with similar results.

The growth of all these strains was prevented by penicillin in concentrations of 0.4 Oxford units and upwards, in 3.5 ml. (0.11 Oxford units per ml.). With lower concentrations, variation of sensitivity to the drug was apparent. Since leptospira multiply more slowly than such bacteria as staphylococci, inhibition of growth compared with controls first becomes noticeable only after about three days' incubation. It was noticed that cultures which were inhibited after three days showed no further sign of growth when examined at intervals up to 14 days.<sup>1</sup> Penicillin thus either kills the leptospira or maintains its activity against them for one to two weeks at 32°C. It was also found that penicillin cultures, which appeared to be multiplying during the first three days, might show marked diminution in numbers when examined at the end of a week.

This possible lethal, as opposed to inhibitory, effect was examined in another experiment. A well-grown 7-day mass-culture of leptospira was distributed in 9.5 ml. amounts in test-tubes, and 0.5 ml. of serial dilutions of penicillin, diminishing by 50 per cent were added. The final series contained 240, 120,

down to 0.5 Oxford units in 10 ml. The mixtures and controls were incubated at  $24^{\circ}\text{C}.$ , and were examined at intervals. As before, counts were made of the number of living motile leptospiras in standard microscope fields.

It was found that concentrations of less than 15 Oxford units of penicillin in 10 ml. had no apparent effect on the leptospiras. After 12 days' incubation the number of living leptospiras was greatly diminished in higher concentrations, but the organisms were not entirely eliminated after three weeks' contact with penicillin. No abnormal morphological forms were noted, such as occur when staphylococci are acted on by penicillin.

This lethal effect of penicillin on leptospiras, associated with lysis and destruction, is similar to the action on staphylococci described by Rantz and Kirby (1944).

A series of animal injections was planned to test: (1) whether infection of guinea-pigs by virulent leptospiras could be prevented by the simultaneous administration of penicillin; (2) whether the course of the established disease could be affected by giving the drug once the symptoms (fever or jaundice) had become apparent. Before these experiments were carried out, Heilmann and Herrell (1944) reported that large doses of penicillin administered at frequent intervals, beginning shortly after infection, will protect guinea-pigs against virulent leptospiras. The aim of this work differed from our own, so preliminary experiments were undertaken on the lines originally laid down.

I found, however, that guinea-pigs inoculated intraperitoneally with virulent *L. ictero-haemorrhagiae*, and given 60 units of calcium penicillin (also intraperitoneally) immediately thereafter and again 48 hours later, died in about the same time



as untreated controls. This dosage corresponds to 14,000 units for a man of 70 kg. I also found that penicillin had no therapeutic effect when treatment was begun after the onset of symptoms i e., on the sixth or seventh day after infection. In these experiments guinea-pigs received, in some cases, doses corresponding to 140,000 units for a man of 70 kg. In further experiments with Dr. J.C. Broom the administration of penicillin was started two to four days before symptoms were expected to appear. The results were irregular, and no definite conclusions could be drawn from them.

We finally carried out one experiment in an attempt to confirm the findings of Heilmann and Herrell. Fifteen guinea-pigs were inoculated intraperitoneally with a virulent strain of *L. ictero-haemorrhagiae*, and treatment with sodium penicillin suspended in an inert oil (arachis oil) was begun 18 hours later in 7 of the animals. Each received 200 Oxford units at 10 a.m. and 5 p.m. and 400 units at 10 p.m. The dose was divided -  $\frac{4}{5}$  was injected subcutaneously and  $\frac{1}{5}$  intraperitoneally. This treatment was continued for 13 days, and on the two following days 400 units were given at 10 a.m. and 5 p.m. Each guinea-pig thus received 12,000 units, corresponding to more than 1,000,000 units for a man of 70 kg.

The result was that 4 of the 8 control guinea-pigs and 6 of the 7 treated animals survived. The seventh treated showed no signs of leptospiral infection at necropsy, and its tissues were not infective when inoculated into other guinea-pigs so that infection with *L. ictero-haemorrhagiae* did not appear to have occurred. This experiment supports the view, therefore, that penicillin, if given early enough, will reduce the death rate

of infected guinea-pigs. In treatment of human beings the use of continuous therapy is a factor which could be expected to increase greatly the efficiency of the treatment and to allow it to be effective in later stages of the infection, by maintaining a steady concentration of the drug in the blood stream. In other ways the experience in guinea-pigs is valuable because human beings and guinea-pigs are very similar to one another in susceptibility to infection by virulent strains of *L. icterohaemorrhagiae*, as well as in morbid anatomy of the disease produced. This work was published (Alston and Broom, 1944).

#### The Wassermann Test in Weil's Disease.

Some writers have reported positive Wassermann tests in serum or cerebro-spinal fluid in Weil's disease and there have been statements that this is one of the acute infectious conditions in which a "false" Wassermann reaction may be found. Costa and Troisier (1916) made a statement to this effect. Sladden (1939) in reporting nine cases of Weil's disease in South Wales stated that the Wassermann test was positive in one serum, weakly positive in two and positive in two specimens of cerebro-spinal fluid. These reactions were absent later in some instances tested.

I have carried out the Wassermann test on sixty of the seventy-nine patients with Weil's disease, whom I have examined bacteriologically, and found them all except one to be negative in the form of <sup>a</sup> routine test, with 3 minimum haemolytic doses of complement, which is done weekly in my laboratory. The single exception was a serum which gave partial lysis only and when it was repeated was a weak reaction of the degree which I report as

suspicious. This patient was a man between 50 and 60 years of age and showed no signs and gave no history of syphilis. The significance of the reaction is therefore in doubt. On the evidence of the remaining tests I do not consider that the Wassermann test is made positive in Weil's disease.

#### Prophylaxis.

In sewer work in London there has been steady application of methods to reduce the volume of infection and to prevent it reaching individual workers. Repairing drains in order to prevent rats reaching the sewers and systematic poisoning of rats twice a year throughout the London County Council's sewers have been mentioned. In addition, means are being applied to reduce the amount of contact with mud, by increasing mechanical means of transport of it in the sewers and from them to the surface. The workmen, of course, wear rubber thigh boots and an effort was made to give them protection to the skin of the hands and forearms by strong canvas gauntlets. These reduced cuts and abrasions but since they were pervious to water made the skin covered by them wet and sodden. Instruction in personal hygiene is given to each flusher employed by the London County Council by a card headed "Precautions against risk of Leptospiiral Jaundice". The card is printed as follows:-

In all "suspect" cases immediately telephone to  
Dr. J. M. Alston,  
Archway Hospital,  
(Archway 3266).

If unable to travel, inform Dr. Alston of your doctor's name and address and telephone number.

#### Instructions.

1. After leaving a sewer, the hands and forearms should always be thoroughly washed with soap and warm water. This should be done before taking

any food or drink.

2. Particular care should be taken to wash thoroughly any cut, scratch or abrasion of the skin as soon as possible, whether the injury was sustained at work or not. An antiseptic should then be applied to the affected part with a clean piece of cloth or cotton wool and the wound protected with a strip of gauze completely covered with adhesive plaster. The wound should be kept covered until it is quite healed.

A suitable antiseptic, gauze and adhesive plaster are supplied for use.

3. Avoid rubbing nose or mouth with the hands during work.
4. Keep this card in a safe place and whenever you go to your doctor or to a hospital on account of illness, show the card and make sure that those attending you know your occupation.

#### Information for the General Practitioner.

Leptospirosis Ictero-haemorrhagica or Weil's disease, generally commences as a febrile illness with varying degrees of the more characteristic features, muscular pains and tenderness, congestion of the conjunctiva, haemorrhages of skin and mucous membranes and jaundice. Jaundice is often absent throughout the illness and is not expected during the first few days.

The urine usually contains albumin and casts and a polymorphonuclear leucocytosis is the rule.

In the early stages, leptospirosis is often mistaken for influenza, pneumonia, tonsillitis, rheumatic fever or nephritis and later for catarrhal jaundice, gall-stones, etc. The diagnosis must be confirmed by special laboratory tests and it is most advisable to send suspected cases without delay into hospital for investigation and for treatment, which may include the use of an anti-serum.

I advised on the form of these instructions. I have frequently been told by a sewer-man's doctor of the commencement of an illness which has proved to be Weil's disease, because my name and the address of my laboratory are given on the card. In this way I have found three anicteric cases and have hastened the admission to hospital of some seriously ill men.



Prophylactic use of vaccines of *L. ictero-haemorrhagiae* was contemplated for London sewer-men. The project was not pursued for a number of reasons. A summary of the relevant literature did not show any consistent results of such vaccination and it was reported that some moderate or severe reactions occurred in the vaccinated (Inada, 1922; Wani, 1933). The risk of Weil's did not amount to more than 1 per cent per year for the sewer-men as a whole and, since the risk was always present, it would be difficult to reduce that figure substantially by vaccination alone. It would not be practical to give the necessary course of vaccination before work began in the sewers and since the chance of infection is very much greater in the first year of work than in any other, there was a danger of failing to protect the men during the early part of the time. It would be possible, also, that the workers might consider that vaccination had caused or precipitated the infection in some cases. I knew that natural immunisation occurs from my experiments on protective antibodies in the blood of sewer-men who had not suffered from jaundice, and it was considered that not enough benefit would be gained in return for the expense and for constantly drawing the attention of the men to the disease. There was a big chance in fact of causing discomfort in the process of vaccination and disappointment in its results.

such alternative might occur if it were realised that infection was produced in many circumstances not connected with employment and if it were possible to detect in many individuals whether employment were to blame or not. My experience has suggested the justification of regarding the disease as one for which vaccination may justly be claimed.

Weil's disease is notifiable in Scotland, but not in

WEIL'S DISEASE AND THE WORKMEN'S COMPENSATION ACT, 1935.

In 1936, I prepared for a Departmental Committee of the Home Office a memorandum on Weil's disease in relation to its occupational risk in sewer-men and gave oral evidence on the subject. The result of the Committee's report to the Home Office was that the disease was scheduled from April, 1940, as an industrial disease under Section 43 of the Workmen's Compensation Act, 1925, subject to special conditions. These conditions are that in non-fatal instances the workman or someone acting on his behalf shall produce bacteriological or serological evidence in support of his application for compensation. In fatal cases, if such bacteriological or serological evidence has not been obtained during life or after death, the dependents would (in default of agreement with the employer) have to produce such general evidence as will satisfy the Court that death was caused by the disease. It should be remembered that an essential condition for scheduling the disease in this way is that the infection is comparatively rare and the circumstances in which an applicant would be exposed to risk of infection would usually be confined to his occupation or could be ascertained with fair certainty. The disease might become unsuitable for retention on the Schedule in the light of increased knowledge or a change in conditions; such alterations might occur if it were realised that infection was produced in many circumstances not connected with employment and if it were possible to detect in many individuals whether employment were to blame or not. My experience has supported the justification of regarding the disease as one for which compensation may justly be claimed.

Weil's disease is notifiable in Scotland, but not in

England or Wales.

In Queensland, Weil's disease was made a compensatable disease under the Workers' Compensation Act in 1934 (Cotter 1936).

LEPTOSPIROSIS IN THE BRITISH ISLES.AN EPIDEMIOLOGICAL AND BACTERIOLOGICAL STUDYPART II LEPTOSPIROSIS CANICOLA.HISTORICAL.

Infection of dogs by *L. ictero-haemorrhagiae* has already been mentioned on the authority of, among others, Courmont and Durand (1917) and Okell et al. (1925). The excretion of the leptospira in the urine of dogs and the relationships to one another of infections in dogs, rats and human beings (e.g. Krumbein and Fieling, 1916) have been commented on. It gradually emerged that dogs are susceptible to infection by another leptospira. This was finally proved in 1931 when Klarenbeek and Schüffner (1933) isolated from the urine of a dog in Utrecht a leptospira which differed serologically from other known species.

The new leptospira was called the Roesel strain; an anti-serum which was made against it agglutinated it to an end titre of 1 in 300,000 and agglutinated *L. ictero-haemorrhagiae* (Zaan strain) to 1 in 300, *L. hebdomadis* to 1 in 30, but did not agglutinate *L. grippo-typhosa* or *L. rachmat*. The serum of a patient suffering from an infection with *L. ictero-haemorrhagiae* agglutinated the Wijnberg strain of *L. ictero-haemorrhagiae* to 1 in 100,000, the Roesel strain to 1 in 1,000 and *L. rachmat* to 1 in 100. The Roesel strain killed two guinea-pigs in 6 and 8 days and guinea-pigs were protected from infection by Roesel antiserum. Klarenbeek and Schüffner considered that spread of this strain to man was possible. Later, they named the Roesel strain *L. canicola* (Schüffner, 1934).

Earlier, Lukeš (1924) had seen a spirochaete in the kidneys of nine dogs; he named the organism *Spirochaeta melanogenes canis* and transmitted the infection from infected



tissues to experimental animals. Since the organism was lost it is not possible to know whether he was dealing with *L. canicola*. Some of the dogs studied by Okell et al. may have been infected with *L. canicola* rather than *L. ictero-haemorrhagiae*; this possibility arises from the more acute, haemorrhagic course of the disease in these dogs and the lower virulence in guinea-pigs of the infection caused by their tissues compared with tissues of others. Similarly, Kouwenaar and Wolff (1930) found that 6 per cent of healthy dogs in Sumatra showed leptospiras on culture from the kidneys and these strains were weakly virulent for guinea-pigs and appeared different from the classical Weil's strains.

There has been gradual clarification of the clinical effects of the two species of leptospiras on dogs. The opinion is becoming accepted (Klarenbeek, 1938; Wirth, 1939; Mills, 1948) that in general, *L. ictero-haemorrhagiae* causes jaundice and sometimes severe nephritis with uraemia; in a small minority, nephritis without jaundice occurs. *L. canicola*, on the other hand, causes jaundice uncommonly, and nephritis with uraemia in about half the cases. The uraemic infections with haemorrhage and, often, severe gastro-enteritis have been known in the past as dog-typhus, ulcerative stomatitis, haemorrhagic gastro-enteritis or Stuttgart disease and are all, or nearly all, due to *L. canicola*. Infection with either leptospira may cause mild symptoms or be inapparent.

*L. canicola* is indistinguishable from *L. ictero-haemorrhagiae* by morphology and in cultural requirements and characters. The two species differ in virulence to various

animals and in antigenic structure, as will be described later.

The viability of the two species outside the animal body and their susceptibility to acid in urine appear to be similar - as judged by experience with isolation of *L. canicola* in culture - although specific experiments to test these factors concerning this species do not seem to be recorded. *L. canicola* is susceptible to penicillin and streptomycin, as will be mentioned more fully in another section.

L. canicola in Dogs.

So far, *L. canicola* has been found to occur naturally only in dogs and man. The occurrence in dogs is so much the commoner, and the infection of human beings is so nearly a direct one from dogs that the relation of *L. canicola* and dogs will be dealt with in detail first.

Infection of dogs by *L. canicola* has been discovered by culture of urine, of kidney or other tissues or by serological tests of agglutination or agglutination and lysis. There is a difficulty in interpreting some serological results with comparatively low end-points in these tests and, in addition to discounting paraspecific reactions due to infection by *L. ictero-haemorrhagiae*, no agreement has been reached of what titre is necessary to indicate former infection or a carrier state without active disease. General opinion seems to point to 1 in 30 as a necessary degree of reaction.

Following their isolation of *L. canicola*, Dutch workers have established a pre-eminence in detecting infection by the species in dogs and men.

Walch-Sorgdrager (1939, p.319) recorded that of 414 dogs in Amsterdam which were believed to be suffering from leptospirosis from 1932 to 1937, 166 were diagnosed serologically as infections by *L. canicola*, 107 by *L. ictero-haemorrhagiae* and the serum of 22 dogs reacted equally with both leptospiras and a diagnosis could not be made between them. (It may be noted that some small inconsistencies of dates and additions seem to occur in this portion of a monograph which, as a whole, is very full, very accurate and very well written). In Utrecht, Klarenbeek (1938) found, from

1933 to 1937, 182 dogs with leptospirosis; 94 were diagnosed serologically as infections by *L. canicola*, 57 by *L. ictero-haemorrhagiae*, 12 showed similar reactions with both antigens and 19 were not tested. Therefore, 58 per cent of those tested were infections by *L. canicola*, 35 per cent by *L. ictero-haemorrhagiae* and 7 per cent were indeterminable between the two. In 1940, Beuvery-Asman reported that he detected by serological tests in Holland in that year 57 infections by *L. canicola* and 56 by *L. ictero-haemorrhagiae* among 280 dogs.

In Germany, Uhlenhuth and Zimmermann (1936) showed that <sup>per cent</sup> in Freiburg 15 to 20/out of 90 dogs gave serological tests to *L. ictero-haemorrhagiae* or *L. canicola* - two thirds to the latter. Infections were mostly latent at the time that the animals were tested. Dahr (1937) tested the serum of 200 dogs in Cologne; 7 were positive with *L. ictero-haemorrhagiae* in titres from 1 in 20 to 1 in 100; 9 were positive with *L. canicola* from 1 in 50 to 1 in 350. Three of these last reacted to 1 in 160 (one serum), or 1 in 350 (two sera) and were negative with *L. ictero-haemorrhagiae*, while conversely one serum reacted to 1 in 100 with *L. ictero-haemorrhagiae* and was negative with *L. canicola*. As a result of this survey Dahr thought that the risk to man of infection by *L. canicola* was small.

In Vienna, Wirth (1937) injected two whelps with the urine of a dog suffering from infection by *L. canicola* and produced a mild illness and leptospiruria in the experimental dogs. By contrast, injection of the kidney tissue of canal rats, containing *L. ictero-haemorrhagiae*, caused in whelps severe and fatal infections of jaundice.



In Antwerp, Van der Walle (1939) found a high proportion of infected dogs, for 44 of 100 gave serological evidence of leptospiral infection, with *L. canicola* twice as frequent as *L. icterogenes* (syn. *L. ictero-haemorrhagiae*). He isolated a strain of *L. canicola* and found it weakly virulent for guinea-pigs and for two young dogs, which had only slight conjunctivitis after intraperitoneal injection.

In Denmark, Brammer et al. (1938) found serological evidence of an infection by *L. canicola* in a dog and isolated the organism from its urine. Petersen (1944a) quoted findings of his own and of others that the infection was widespread among dogs in big towns in Denmark.

Gsell and Kanter (1945) stated that in one year (1944-1945) 114 cases of leptospirosis *canicola* were seen in a veterinary clinic in Zurich.

In Norway, Grelland found leptospiras in the kidney and a strong serological reaction to *L. canicola* in the blood of a dog which had infected a woman (Grelland, 1946).

Mochtar and Collier (1939) found in Batavia one dog in 152 with a high titre of agglutination (1 in 1,000) for *L. canicola*.

Kolochine-Erber et al. (1945) isolated a strain of *L. canicola* from a dog in France. In the same year in that country Senthille et al. (1945) reported a dog, its bitch and their two puppies which all showed high titre of agglutination of *L. canicola*.

Snapper et al. (1940) reported the infection of dogs with *L. canicola* in Northern China.

A considerable amount of investigation of leptospirosis of dogs has been made in U.S.A. Meyer et al. (1939) investigated

87 dogs in California and found that 38 had haemorrhagic enteritis or Stuttgart disease and 42 had "yellows" or infectious jaundice. From those with the former syndrome they isolated 11 strains of *L. canicola*.

Greene (1941) tested the serum of 368 dogs in Los Angeles and found that 29 per cent of them gave a significant reaction with *L. canicola*; these comprised 19 per cent of the females and 30 per cent of the males, that were tested. Raven (1941) found in Pennsylvania that rural dogs showed more serological evidence of leptospirosis than urban dogs - in 38 per cent and 28 per cent respectively. In both cases more sera were positive with higher titres when tested with *L. canicola* than with *L. ictero-haemorrhagiae*. In Denver, Colorado, Rosenbaum (1946) discovered a dog whose serum agglutinated *L. canicola* to 1 in 100,000 and *L. ictero-haemorrhagiae* to 1 in 1,000 and was the cause of a human infection. In 1937, Jungherr wrote of an outbreak of jaundice in a kennel of pointers on the shore of Long Island Sound. He saw leptospiras in tissues, cultured them and found that they had only low virulence for guinea-pigs. It is open to question whether they were *L. canicola* or not.

In South America, Savino and Rennella (1944) isolated six strains of *L. canicola* from 390 dogs; among 317 dogs they found that 25 per cent agglutinated *L. canicola* significantly.

The evidence about *L. canicola* in the British Isles will be dealt with in a later section.

It is clear that this species is widely distributed throughout the world, among dogs. The percentage of dogs in whom the organism has been revealed in different surveys varies widely

from place to place being affected, for one thing, by the fact of whether healthy dogs at random, sick dogs in general or dogs suspected of leptospirosis were the subject of the survey, but it appears that from 30 to 40 per cent of apparently healthy dogs may show antibodies in sufficient degree to point to former infection by *L. canicola*.

So far as observation has gone, all breeds of dogs seem liable to infection (Meyer et al. 1939), but it has not been found naturally in any other species of animal. Beuvery-Asman (1940) and Greene (1941) found no reactions in testing the serum of 50 and 100 cats respectively.

Although jaundice is exceptional in dogs infected with *L. canicola*, in some groups of cases it has been relatively common. For instance, Meyer et al. (1939) recorded that they found it more frequently in California than it had been found by workers in Holland.

## EPIDEMIOLOGICAL FEATURES OF THE INFECTION IN DOGS.

### Sex and Source of Infection.

It has been the general but not quite unanimous experience that male dogs are *oftener* infected than female. This was the experience, for example, of Meyer et al. (1939) in California where the ratio of male to female was 67:18 for fatal infections, of Raven (1941) in Pennsylvania, of Van der Walle (1939) in Belgium, of Klarenbeek and Winsser (1938) in Holland. Beuvery-Asman (1940), however, recorded almost equal percentages of infection in the sexes.

The reason for this difference between the sexes lies in the fact that the source of infection is almost entirely dog's urine in which the organism may be excreted for weeks or months after infection commences. Secondly, the habit of dogs and especially male dogs of licking and smelling the genitals and the excreted urine of other dogs explains the usual mode of transfer and the greater risk to male dogs. Besides this method of infection, of the mouth or nose, contamination of food or drink with the urine of other dogs is a possible source of infection and, uncommonly, dogs bathing together in fresh water may infect one another.

### Age.

The evidence about the commonest age at which infection occurs and the relative susceptibility of younger contrasted with older dogs is not yet complete. Statements are found frequently in the literature to the general effect that infection is commoner in older animals but the evidence does not clearly support that view. The greatest bulk of information is about serological



tests of healthy dogs which have recovered from earlier infections. For example, Raven (1941) gave the ages of 50 dogs, in and around Philadelphia in which serological diagnosis of past infection was made, as 19 per cent for dogs up to 1 year of age, 25 per cent for those 1 year to 3 years and 46 per cent for 3 years to 10 years. This indicates that nearly one-fifth of the dogs had been infected before they were one year old and since the agglutination reaction remains positive for months or years it is not surprising that the percentage of reactions increases with age. Meyer et al. (1939) stated that the disease was rare in California under the age of one year.

Walch-Sorgdrager (1939) reported that "in Klarenbeek's Clinic there were 81 dogs with leptospirosis (between 1934 and 1937) of which 13 were less than a year old, 51 1 to 5 years old, 17 over 5 years old". The percentage for each of the three age periods would be, therefore, 16, 63 and 21 respectively.

Van Thiel (1948, p.140) stated that "the very high titres against *L. canicola* being mainly found in dogs from 2 to 4 years old the susceptibility to the acute disease is most prominent in this period. In older dogs only weak titres were found, probably remnants of a previous infection (a weak titre may persist for many years)".

Natural infection of puppies has been found at or soon after birth (Senthille et al. 1945; Roos et al., 1937). Puppies can be infected experimentally (Walch-Sorgdrager, 1939) and in such young dogs jaundice occurred in ten out of eleven (Klarenbeek and Winsser, 1938).

Since the risk varies from place to place and time to

time it would seem that young dogs are clearly susceptible, and there is at any rate no evidence - rather the contrary - that the first year of life is a very resistant age.

#### Seasonal Incidence.

Klarenbeek (1938) compared the seasonal incidence in dogs of infection by *L. ictero-haemorrhagiae* and *L. canicola*. In the former, 29 of 57 infections (51 per cent) were in the months of September, October and November, but only 35 of 94 (37 per cent) of the latter were in those three months; the other 59 cases were distributed irregularly in all the other months of the year. This is not such clear evidence of concentration in the second half of the year as will be seen with human infections. Klarenbeek and Winsser (1938) reported a series of 35 infections of dogs by *L. canicola* in 1935 and 1936 in which one case or more occurred in each month of the year except September, the largest numbers - 5 and 4 respectively - occurring in February and October.

#### Case Fatality Rates.

In all infectious diseases, in man or in animals, the mortality rate due to a disease in the population as a whole, and the case fatality rate in individuals known to be infected, greatly depend on the success with which mild instances of the infection are sought. The only exceptions to this are in the diseases in which no fatalities have been attributed to the infection. This is almost the case with *L. canicola* in man, but clearly not in the dog.

Dhont et al. (1934) calculated the case fatality rates for severe infections in dogs by *L. ictero-haemorrhagiae* and *L.*

canicola. They stated that when the leptospirosis produced jaundice and an acute course of disease, *L. ictero-haemorrhagiae* was found to be the infecting agent and the case mortality rate was 50 per cent. In an equal number of dogs, azotaemic uraemia was found; *L. canicola* appeared to be the infective agent in 90 per cent of these and in this nephropathic form of the disease - whether it was acute or chronic - the mortality was 81 per cent.

Klarenbeek (1938) writing more generally of infections by *L. canicola* contrasted a mortality rate of 51 per cent for *L. ictero-haemorrhagiae* with 41 per cent for *L. canicola*.

Only 3 types of leptospirosis. Ten out of 17 died in the second passage and were in the subsequent passages. After the third passage, the period between inoculation and death did not appear to be definitely related any further. From observations on the recovery of *L. ictero-haemorrhagiae* from blood and urine respectively, Falck-Schlegel showed that (as is the common belief) urine reduces the number of the virulence of leptospires for guinea-pig inoculation, but when all urine is used for this in the inoculation of results of infecting guinea-pigs with *L. canicola* in urine, *L. canicola* is much less pathogenic than *L. ictero-haemorrhagiae*. The same worker also reported that *L. canicola* caused little jaundice in guinea-pigs in Holland and that it did not cause slight and occurred in all passages of the particular strain. Meyer et al. (1939) found that young guinea-pigs susceptible to *L. canicola*, but not fatally so.

Falck-Schlegel found experimentally that rats were very little affected by inoculation of *L. canicola* and were not considered carriers of the infection. In the case of mice, some were experimentally infected by a single strain of which the virulence

INFECTION OF EXPERIMENTAL ANIMALS.

It has been the general experience that *L. canicola* is much less virulent towards guinea-pigs than *L. ictero-haemorrhagiae*. It was noted above, however, that in the original experiments with the Roesel strain of the species, Klarenbeek and Schüffner<sup>1933</sup> found that it killed two guinea-pigs in 6 and 8 days. Walch-Sorgdrager (1939) has recorded extensive experiments which are in line with general experience. She found that <sup>of</sup> 37 guinea-pigs inoculated with canine or human urine containing living *Leptospira canicola*, only 27 became infected; 22 of them survived the first passage, only 5 dying of leptospirosis. Ten out of 17 died in the second passage and more in the subsequent passages. After the third passage, the period between inoculation and death did not appear to be definitely reduced any further. From observations on the recovery of *L. ictero-haemorrhagiae* from blood and urine respectively, Walch-Sorgdrager showed that (as is the common belief) urine reduces the number or the virulence of leptospiras for guinea-pig inoculation; but when allowance is made for this in the consideration of results of infecting guinea-pigs with *L. canicola* in urine, *L. canicola* is much less pathogenic than *L. ictero-haemorrhagiae*. The same worker also recorded that *L. canicola* caused little jaundice in guinea-pigs in Holland and when it did occur it was slight and occurred in all passages of the particular strains. Meyer et al. (1939) found very young guinea-pigs susceptible to *L. canicola*, but not fatally so.

Walch-Sorgdrager found experimentally that rats were very little affected by inoculation of *L. canicola* and were not made carriers of the species. In the case of mice, some were temporarily infected by a single strain of which the virulence



had been raised by passing it through a guinea-pig. Larson (1944) reported that *L. canicola* does not cause symptoms in mice. Cats and kittens were found by Walch-Sorgdrager and by Klarenbeek and Winsser (1938) to be slightly and almost equally susceptible to *L. ictero-haemorrhagiae* and to *L. canicola*. With both species of leptospiras Walch-Sorgdrager (1939, p. 332) found that the organism appeared in the peritoneal fluid of kittens after injection elsewhere and one kitten became a carrier of the organism with each species.

An important discovery was made by Morton (1942) that the hamster (*Cricetus auratus*) can be used for isolation of *L. canicola*. He reported that the species ~~does~~ not kill young hamsters, 3 to 5 weeks old, but the blood cultures taken from them 48, 72, and 96 hours after inoculation of infected material are positive. He found that, by contrast, hamsters of the same age are killed by *L. ictero-haemorrhagiae* in 5 to 8 days from the time of infection. This discovery has been used widely for isolating *L. canicola*, and it has been found later that at least some strains of *L. canicola* kill young hamsters (Larson, 1944) unless they are protected by penicillin (Larsen and Griffiths, 1945) or by serum (Larson, 1944; Laurent et al., 1948).

Reference has already been made to experimental infection of adult and young dogs.

NATURAL INFECTION OF DOGS BY *L. CANICOLA* IN THE BRITISH ISLES.

The knowledge of canine infections by *L. canicola* in the British Isles has been investigated by only a few workers.

In 1942, I examined for agglutinins to *L. canicola* the serum of 100 dogs kindly sent to me from the Wellcome Physiological Laboratories, Beckenham, Kent. Of these, 34 showed agglutination of *L. canicola* in dilutions of 1 in 30 or 1 in 100. The dogs were of different ages, breeds and sex and were killed after being used for experiments on vaccine against the virus of dog distemper. None was known or suspected to be suffering from leptospirosis. I considered that the results indicated past infections by *L. canicola*. At the same time, I endeavoured to detect *L. canicola* in the kidneys of many of these dogs by microscopical examination and by injection into young guinea-pigs of crushed kidney tissue from groups of dogs. Since I did not detect leptospira of any species by either method, I did not attempt to publish the serological results.

R.D. Stuart (1946) reported that 40 per cent of 100 house dogs in Glasgow had been infected with *L. canicola* as judged by agglutination reactions of their sera reaching dilutions of 1 in 10 to 1 in 3,000. These dogs were an unselected group of many breeds, more male than female and relatively old. In the animals of which the sex was known a higher percentage of males (47) than of females (27) was found positive; the percentage of positives increased with age from 25 in the first year of life to 55 in the period of 5 to 9 years. In a small group of 14 kennel dogs the serum of none was found to react with *L. canicola*. Cross agglutination with *L. ictero-haemorrhagiae* was found in some dogs, and

Stuart concluded that in addition to the infections by *L. canicola*, 6 of the 100 house dogs and 4 of 14 kennel dogs showed evidence of infection by *L. ictero-haemorrhagiae*.

J.C. Broom (unpublished) allows me to refer to similar results which he obtained in dogs in London during the Second World War. From 384 dogs he found 89 (23 per cent) of which the serum agglutinated *L. canicola* at an end-point of 1 in 10 to 1 in 3,000; 52 of the reactions showed an end-point of 1 in 10, 1 in 30 or 1 in 100. By contrast, only 8 animals gave a serological reaction with *L. ictero-haemorrhagiae* at 1 in 30 or higher dilution.

In 1947, Joshua and Freak reported the treatment by penicillin of six dogs in London which had been diagnosed with the serological assistance of J.C. Broom as suffering from infection by *L. canicola*. Five recovered (including the only one in which a low titre of agglutination did not so strongly confirm the diagnosis as in the remainder) and one died. In four of the survivors recovery was considered to be complete, but in the other, permanent kidney damage seemed likely. In the fatal instance, post-mortem examination showed inflammatory changes in kidneys, liver, pleurae and pericardium, with recent acute pneumonia as the actual cause of death. The writers of this paper considered that these results were very successful and that the outcome of treatment with penicillin depended on the degree of tissue damage at the time the treatment began. They surmised that the response to penicillin is so spectacular that the use of the drug as a diagnostic aid in suspected cases is justified.

Mills (1948) gave a good review of clinical aspects of

canine leptospirosis due to *L. ictero-haemorrhagiae* and *L. canicola*. She identified, with the serological aid of R.D. Stuart, 19 instances of leptospirosis among 30 dogs suspected of the infection; in 14 the disease was considered due to *L. canicola* and in the other 5 to *L. ictero-haemorrhagiae*. Seven of the 14 infections by *L. canicola* were considered acute or sub-acute and the others long-past infections. Five of the animals infected by *L. canicola* showed predominantly uraemic symptoms; jaundice was not recorded in any of the dogs. Among 11 healthy dogs, 3 gave a reaction with *L. canicola* to a titre of 1 in 300. Along with this work, Jennings (1948) made post-mortem examination of 4 dogs, two of which had died from each of the <sup>species</sup> leptospira. In one dog which died of infection by *L. canicola* the changes from healthy conditions, which he found, included acute inflammation of the stomach with ulceration and haemorrhage of the fundus; a varying degree of enteritis and haemorrhage throughout the intestines; dark purple colour and great congestion of the spleen; the liver was congested and showed four circular areas of necrosis; the kidneys were small and contracted and the capsules were adherent; the cortex of the kidneys was rough, granular and mottled. Histologically chronic nephritis was found. In the second dog which died of infection by *L. canicola*, similar conditions were found except that the kidneys showed more acute nephritis.

In 1943, Lovell gave a general review of infection of dogs by *L. ictero-haemorrhagiae* and *L. canicola*, without new observations.



### HUMAN INFECTIONS BY *L. CANICOLA* IN THE WORLD GENERALLY.

The first two human infections by *L. canicola* were reported by Dhont, Klarenbeek, Schüffner and Voet (1934). One was of a boy of 16 years in Holland who had been in contact with rats and a fox-terrier. The illness was mild with transient jaundice of the sclerae; both the boy and the dog showed clear serological evidence of infection by *L. canicola*. The second patient was a man of 51 years and a strain of *L. canicola* was isolated from him. Nine more infections were found in Holland by 1937, (Schüffner, Kotter and Schultz, 1935; Schüffner and Walch-Sorgdrager, 1937; Roos, Walch-Sorgdrager and Schüffner, 1937). In 1938, Brammer et al. reported an infection in Denmark and since then active infections have been found in Germany, United States, ~~Rosetox~~, China, France, Norway, Great Britain, Porto Rico, Switzerland and Argentina in approximately that order chronologically. Table 16 gives a list of these countries with, for each, the date of the first recorded infection, the author of the primary and of some other records and the total number yet reported.

In the Belgian Congo, Van Riel (1939) found 32 patients infected by a leptospira which is serologically related to *L. canicola* but which appeared to be a new species. These infections have not been counted for the present purpose.

The records of human infections by *L. canicola* have been made unnecessarily complicated by multiple reports of the same patients and by inaccurate quotation. The most glaring example of the first fault concerns three French patients Monsieur A., Madame A. and Madame P. These people were infected by a family of four dogs. The infections of the dogs, the man and the two

women are recorded fully in each of two publications by Senthille, Bayo and Kolochine-Erber (1945) and Kolochine-Erber and Colombier (1945a). In addition, the same events are reported piecemeal in three papers by Bolgert, Kolochine-Erber and Noël (1945), Bolgert, Kolochine-Erber, Noël and Sigwalt (1945) and Kolochine-Erber and Colombier (1945<sup>a</sup>). Another example of redundant publication is shown in the two reports, without reference to each other, of a woman treated in the Allgemeinen Krankenhaus, Hamburg-Barmen. This illness was recorded by Stödter (1938) and Tetzner (1938). Based on this duplication, Tievsky et al. (1944) and Gaillemin (1946) have stated that two patients are involved, one in Germany reported by Stödter and another in Austria reported by Tetzner.

Similarly, Gaillemin (1946) stated that at the time of 1938, five instances had been recorded in England. This is a mistake arising from confusing R.D. Stuart's (1938) record of a past infection by *L. canicola* in a tripe-dresser with his finding in five other women of the same occupation who had antibodies which signified previous infection by *L. ictero-haemorrhagiae*.

Minkenhof (1948) gave confusing information about infections in Holland. He stated that 40 infections "have been reported" for the Netherlands, and then recounts that "in the Institute of Tropical Hygiene in Amsterdam 49 human cases of this disease were diagnosed between 1933 and the autumn of 1947". Since the infection has been diagnosed at more than one centre in Holland we are left quite uncertain of what number of the 40 first mentioned are also included in the 49.

Professor Charlotte Ruys, Amsterdam, has informed me (September, 1948) that (in spite of the doubt left in Minkenhof's paper) the total number of human infections by *L. canicola* so far recognised in Holland is 52.

Table 16 shows the countries in which well authenticated infections by *L. canicola* have been reported, the names of the first and some of the later authors and the total of infections for each country. The total for the world, including the British Isles is 145.

ROUTE OF INFECTION AND INCUBATION TIME IN HUMAN BEINGS.

There has not been so much observation and speculation on the route of infection by *L. canicola* as in the case of *L. ictero-haemorrhagiae*. Schüffner et al. (1935) believed that a man and his daughter had been infected by food contaminated by dog's urine, because the illness began in both patients at the same time. Such a double, or a multiple, infection is rare. A few infections occur which are attributed to bathing; Walch-Sorgdrager (1939 p.336) recorded infection of a man and his daughter who had bathed together and were taken ill on the same day as one another; and an infection occurred after bathing in the Thames (Baber and Stuart, 1946). Cuts and abrasions of the skin have not been emphasised in this infection and it is left to be surmised that, as with *L. ictero-haemorrhagiae*, the organism can enter by small abrasions or through the wet skin, through the mouth and throat if infection is conveyed to them by fingers or food, or by the mucous membrane of the mouth or throat or conjunctiva in bathing. With *L. ictero-haemorrhagiae* an hypothesis was made that meningitis was especially likely when infection entered by the mouth or throat. If this were applied to leptospirosis *canicola*, in which meningitis of similar degree is much more frequent, it would suggest that *L. canicola* enters by the mouth more frequently than *L. ictero-haemorrhagiae*. The question requires more evidence.

There is very little accurate knowledge of the incubation time of the infection, since it has nearly always followed contact with a dog for a long or indefinite time.



INCIDENCE AS REGARDS SEX, AGE, SEASON AND OCCUPATION.

The incidence of the disease in the sexes is almost equal. Petersen (1944a) stated that of 47 patients in Denmark 24 were male and 23 female; from records of 21 other patients in most of the countries in which the disease has been recognised I have found 14 men and 10 women, making with Petersen's series 38 men and 33 women.

The infection has been found at a wide range of ages from 10 to 63 years.

In most countries the disease occurs chiefly in the second half of the year. Petersen 1944(a) found that of 47 infections in Denmark during the years 1934 - 1943; the onset was in the month of October, November or December 33 times. Minkenhof (1948) stated that all of 17 instances in Amsterdam between autumn 1946 and the end of 1947 occurred by month as follows - 1 in July, 2 in August, 5 in September, 4 in October and 5 in November. The six acute infections recognised so far in the British Isles began in April, once, July once, September three times and October once. This seasonal incidence is more pronounced than occurs with infections of dogs by *L. canicola* (as noted above), but it is similar to the seasonal incidence of classical Weil's disease in human beings. If the incidence of infections by *L. canicola* were more clearly in the coldest months of the year rather than the last four months the fact of dogs being more in the house in winter time might be suggested, but I do not know the reason for the concentration of infections that occurs.

Occupation is not so predominant a factor as with *L. ictero-haemorrhagiae*. The circumstances of infection are usually

domestic, with the risk increased for either sex by caring for sick dogs, for women in cleaning the floor, for children playing with <sup>dogs</sup> or taking up infection from the floor. Professional risks have occurred in one Swiss and two American veterinarians, who were infected after attending dogs which were ill of the disease (Gsell and Kanter, 1945; Meyer et al. 1938). Similarly two laboratory workers became infected at Peiping Union Medical School, China, after handling dogs' urine every day for some time (Snapper et al. 1940). Infection of a maid servant in Denmark (Brammer et al. 1938) is in the same category of occupational risk.

### SOME CLINICAL FEATURES.

An attempt is not made to describe the disease clinically, although details of the course of the disease in five British patients are given later. In the present section, comment will be made on jaundice, meningitis, conjunctivitis or other signs in the eye, nephritis and skin rash and comparison made of the occurrence of these in leptospirosis canicola and classical Weil's disease.

Jaundice is absent or inconspicuous in almost all human infections by *L. canicola*. Walch-Sorgdrager (1939) stated that it was present (in the sclerae only) in 2 of the first 12 human instances known. Petersen (1944 (a)) found it once in a definite form and three times in the sclerae only, in 47 patients; Minkenhof (1948) recorded it as present, in unspecified degree, in 6 of 49 patients. These amount to 12 times in 108 (9 per cent). In frequency and severity of hepatitis, there is thus a contrast with Weil's disease.

Meningitis is much more prominent in infection by *L. canicola* than by *L. ictero-haemorrhagiae*. It is the most important localised lesion which the infection causes. It was present in 23 of 34 (67 per cent) patients in whom I have found the feature mentioned; it was present in at least 5 of the 6 active infections recognised in the British Isles. When infections are discovered more frequently it is probable that the proportion of meningeal lesions will decrease. There are no records of severe mental or nervous sequelae, but recurrent or persistent headache sometimes follows the illness and I have heard of an unpublished instance in England (Dr. J.C. Broom) in which the patient, who was a physician, attributed troublesome minor fits

to the infection.

Conjunctivitis, iritis, retinitis may occur. I have found that such changes were present in 9 patients out of 22 for whom the state of the eyes was mentioned. The changes are usually of minor degree, and less than frequently occur in Weil's disease.

Nephritis is absent or of slight importance in infection by *L. canicola*. This is a great contrast to Weil's disease.

The first death from the disease occurred in 1947 in Holland. This is referred to by Van Thiel (1948, p.139).

Rash on the skin has been recorded in a few patients studied abroad (Senthille et al. 1945). It was present as a morbilliform eruption in three of four infections reported in England by Laurent et al. (1948).



SUSCEPTIBILITY OF L. CANICOLA TO PENICILLIN AND STREPTOMYCIN.

In 1944, I found that a strain of *L. canicola* (from the National Collection of Type Cultures) was sensitive to penicillin equally with nine strains of *L. ictero-haemorrhagiae* (Alston and Broom, 1944). The method of this test has been described in part I of this thesis in the section describing my own work with *L. ictero-haemorrhagiae*.

Petersen and Schmidt (1945) also found a strain of *L. canicola* susceptible to penicillin in concentrations of 0.33 Oxford units per c.c.

Wylie and Vincent (1947) obtained similar results with penicillin and found also that streptomycin is inhibitory to the species, although rather less so on a basis of weight.

Larson and Griffiths (1945) infected four hamsters with *L. canicola* and saved the lives of three of them by giving 200 units of penicillin twice a day for four days, commencing 66 hours after infection; four hamsters similarly infected which did not receive penicillin died 4 to 8 days after infection.

As has been described more fully in the section on leptospirosis *canicola* in the British Isles, Joshua and Freak (1947) believed that penicillin was successful in treating dogs. If this is confirmed it is important because the disease is widespread and severe in dogs in all countries. Penicillin has been used for human patients (Baber and Stuart, 1946; Laurent et al. 1948) but since the infection is mild and variable in its course the effect of the drug is not yet known.

SEROLOGICAL RELATION OF L. CANICOLA TO OTHER SPECIES OF  
LEPTOSPIRAS.

By serological tests, *L. canicola* shows an antigenic relationship to *L. ictero-haemorrhagiae*, to the Salinem group of leptospiras and to *L. hebdomadis*. This is illustrated in a table of reciprocal reactions of agglutination and lysis shown by eleven species or groups of species, in Walch-Sorgdrager's monograph (1939, p.253). In this, it is shown that a serum prepared against *L. canicola* had an end-point of 1 in 10,000 to 1 in 30,000 with strains of *L. canicola*, end-points of 1 in 30 to 1 in 10,000 with various strains of *L. ictero-haemorrhagiae*, of 1 in 300 to 1 in 1,000 with Salinem strains and of 1 in 30 with *L. hebdomadis*; the serum did not react with *L. grippo-typhosa*, *L. andaman B*, *L. pomona*, *L. andaman A*, *L. bataviae* (Swart v. Tienen group), *L. Akiyami A*. Reciprocal tests of strains of *L. canicola* with anti-serums produced against the species named gave confirmatory results.

The close serological relationship of *L. ictero-haemorrhagiae* and *L. canicola* causes non-specific reactions in the blood of animals or men infected with either species. This is sufficiently marked to need more attention in serological diagnosis than it has obtained so far in this and many other countries, and especially so in the anicteric examples of leptospirosis which might be due to either organism. Gispen and Schüffner (1939) stated that such overlapping reactions were more frequent when the infecting organism was the incomplete (A) type of *L. ictero-haemorrhagiae* than with the complete (AB) type. Broom (1948) discussed this among serological aspects of Weil's disease in human

beings, and gave records of a patient whose serum reacted almost equally with *L. ictero-haemorrhagiae* and *L. canicola* on the sixteenth day of illness; absorption tests showed that the infection was due to *L. ictero-haemorrhagiae* and on the twenty-sixth day of disease the reaction of unabsorbed serum was much higher with *L. ictero-haemorrhagiae* than with *L. canicola*. In the section on infections by *L. canicola* in the British Isles, I mention a test I have made of a serum taken from a patient eleven months after infection by *L. canicola*; the reactions with *L. canicola* and *L. ictero-haemorrhagiae* were then equal, although during the acute stage the reaction with *L. canicola* was the higher, and absorption tests had shown that this species had been the infecting cause of the illness.

In infections of men and dogs by *L. canicola* the end-point of serological tests with the infecting organism may be higher than is found with *L. ictero-haemorrhagiae*. For instance, Senthille et al. (1945) record two human infections with end-points of agglutination of 1 in 2 million and 1 in 5 million. In addition, antibodies may be slower to develop than in classical Weil's disease and if a test is negative it should be repeated until the fifteenth day of the illness.

Van Thiel (1948, p.15) states that the original Roesel strain of *L. canicola* is an incomplete biotype compared with others, such as the Antwerp strain.

HUMAN INFECTIONS BY L. CANICOLA IN THE BRITISH ISLES AND MY  
OWN EXPERIENCE OF THEM.

So far as I know, there are three published accounts of suspected or proved infections of human beings by *L. canicola* in the British Isles. In 1938 Stuart, gave a strongly suggestive evidence of past infection with this species of leptospira in a woman aged 43 years, who was a tripe-scraper in Glasgow. She had kept dogs in her home but could not remember any being ill. Her serum agglutinated *L. canicola* to a dilution of 1 in 40, was "entirely negative" with *L. ictero-haemorrhagiae* and when it was absorbed with *L. canicola* the serum no longer agglutinated that species, but when absorbed with *L. ictero-haemorrhagiae*, agglutination of *L. canicola* was unimpaired. Stuart discussed clearly the likely significance of the agglutination in the light of the cross-absorption test and of the known decrease of agglutination reactions after some instances of infection by *L. ictero-haemorrhagiae* and came reasonably to the conclusion that he had found a past infection by *L. canicola*.

The first instance of the infection recognised in the British Isles, during the acute stage, was recorded by Baber and Stuart (1946). The patient was a boy aged 11 years who entered hospital on September 10th, 1945, with haematuria, malaise, headache and pains in the calves for two days. Twenty-one days previously, he had bathed in the Thames and soon afterwards noticed that his throat was sore. The soreness of the throat passed off, and he again bathed in the Thames, twelve days before entering hospital. The illness was febrile with slight oedema of the eyelids, slight injection of throat, doubtful neck rigidity and obvious haematuria and albuminuria.



On the fourth day in hospital, headache, photophobia, stiffness of the neck and slight blurring of the optic disc were present. Cerebro-spinal fluid was of increased pressure; it contained 690 white cells per c.mm., and 70 per cent of them were lymphocytes and 30 per cent polymorphs. On this day penicillin was given intrathecally and intramuscularly and continued for three days. Improvement occurred after 12 hours of this treatment. Blood was taken on the 9th day of the illness and I found that it agglutinated *L. ictero-haemorrhagiae* to an end-titre of 1 in 1,000. At that time I had not a preparation of antigen of *L. canicola*, although for some time previously I had used one, and therefore I reported the result with *L. ictero-haemorrhagiae* to the hospital which was in Surrey. At the time, from the history that the patient had bathed in the Thames, I thought that the infection was an example of leptospirosis combining meningitic and nephritic signs, due to *L. ictero-haemorrhagiae*. Fortunately, blood four days later was sent to Dr. R.D. Stuart, who by the results on successive sera taken on the 13th day, at the fifth week and at the eleventh week and by cross absorption tests, showed that the infection was due to *L. canicola*. The highest titre found of agglutination of *L. canicola* was by the patient's serum taken at the fifth week when it reached 1 in 30,000. The boy left hospital 3 weeks after admission and he was then quite well. At the end of another six weeks, however, he was admitted again with history of slight haematuria and a headache. He recovered very quickly in hospital and estimation of urea in the blood and of urea-concentration tests gave normal results.

The manner of this infection could only be surmised.

The boy did not possess a dog, nor play with dogs. Probably the infection was acquired while bathing as has been surmised in similar cases previously (Walch-Sorgdrager, 1939).

The third publication on this subject recorded four cases of *Leptospira canicola* infection in England (Laurent, Norris, Starks, Broom and Alston, 1948). These comprised a man and two women who were taken ill within a period of less than a fortnight, in September, 1947 and a man whose illness began in April, 1948. One of the men infected in 1947 lived in the North-East of England and the other three people in London.

Summaries of these four cases (in three of which I had some part in the diagnosis) are as follows:-

#### Case 1.

An apprentice fitter and turner, aged 19 years, living in S.E. London was admitted to hospital on September 15th, 1947, as a case of cerebro-spinal meningitis. He was taken ill suddenly, on September 11th with frontal headache, fever and sweating. Next day his eyes were red and the neck stiff. On the fifth day a pink blotchy rash appeared on the face and chest; no drugs had been taken. His previous history recorded measles and rubella.

On admission. Fifth day of illness. Temperature 100.8°F, pulse-rate 68, respiration rate 22 per min. The patient was a well-nourished young man, conscious, alert and co-operative. The palpebral and bulbar conjunctivae were intensely injected and red, with a little discharge and pronounced photophobia. There was a pink, discrete macular rash chiefly on the chest, abdomen and back, and a little less on the face and extremities. The rash was morbilliform but lighter in colour than that of measles and not irritating. He had no cough, no Koplik's spots, and no redness or ulceration of the buccal mucosa. There was no enlargement of the lymph-glands or spleen and no jaundice. Clinical examination of heart, lungs and abdomen revealed nothing abnormal. Blood-pressure 130/80 mm. Hg; urine, sp.gr. 1.020; no abnormality. There was no urethral discharge and no sore on the genitals. There was some stiffness of the neck on flexion but Kernig's sign was negative and he could kiss his knees. Cranial nerves, fundi, and ears were normal. There was no loss of power or sensation and all the reflexes were normal. Lumbar puncture; fluid clear and colourless with less than 4 cells per c.mm., protein 30 mg. per 100 ml., chlorides 690 mg. per 100 ml., and no organisms.

Subsequent Course. On September 19th (8th day) his temperature was 101.4°F., he was drowsy, his neck was more stiff, he could not kiss his knees, and Kernig's sign was positive. Lumbar puncture now gave a faintly opalescent spinal fluid with 290 cells per c.mm. (mononuclears 50 %); protein 30 mgms. per 100 ml., chlorides 680 mgms. per 100 ml, no organisms and sterile on culture. White-cell count: 6,800 per c.mm., (polymorphs 76.5 %, eosinophils 0.5 %, basophils 1 %, monocytes 1 % and lymphocytes 21 %). Blood culture (twice), Widal test, Wassermann and Kahn tests and Paul Bunnell tests were all negative. X-ray films of the nasal sinuses showed no evidence of infection.

On September 20th (9th day) his temperature was normal and it remained normal afterwards. The rash faded rapidly after that date without leaving any staining of the skin. On October 3rd (22nd day) his eyes were normal, all traces of meningitic signs had disappeared, and no abnormal neurological signs could be detected. He was discharged home quite well on October 20th, the 39th day after the illness began.

A differential diagnosis had to be made between rubella, measles, erythema multiforme, Stevens-Johnson syndrome, and secondary syphilis. The possibility of some bacterial or viral meningitis had also to be considered. Against rubella was the late appearance of the rash, its larger elements, and the absence of enlargement of the lymph-glands. Against measles was the colour of the rash and the absence of staining, the total absence of cough, and the lack of swelling of the eye-lids and of discharge in the presence of such a severe conjunctivitis. Also, he had previously had rubella and measles. The shape and distribution of the skin lesions and the absence of involvement of the buccal mucosa did not suggest either erythema multiforme or Stevens-Johnson syndrome. The rapid subsidence of the eruption and the negative serological tests excluded secondary syphilis. The rash bore no resemblance to any seen in meningococcal infections, and the negative blood culture, the sterile cerebrospinal fluid, and the patient's rapid recovery were against cerebro-spinal fever.

A diagnosis of "aseptic benign meningitis" of virus

aetiology - e.g., poliomyelitis, chorionmeningitis, etc. - was not acceptable as it did not account for two outstanding features, the severe conjunctivitis and the macular rash. The possibility of leptospiral meningitis was then thought of and by a series of agglutination tests of blood taken on the 24th, 50th and 100th days after the onset, a diagnosis of infection by leptospira canicola was established. The details of the serological tests are given below.

The patient had kept a puppy, six months old, just before his illness, but so far as he knew the dog was healthy. Unfortunately this dog was accidentally killed before the patient's infection was diagnosed.

#### Case 2. At Archway Hospital.

A woman, aged 28, was admitted to hospital on September 25th, 1947. She had been quite well until 5 days before admission, since when she had suffered from increasing headache, vertigo and vomiting. Small red spots, which disappeared after 24 hours, had been noticed on the right arm and on both legs.

On admission. Fifth day of illness. Temperature  $101^{\circ}\text{F.}$ , pulse-rate 84, respiration-rate 20 per min. The patient was pale, her tongue was furred, there was well-marked neck rigidity, and Kernig's sign was positive. The eyes, cranial nerves and ears were normal. Lumbar puncture (3 hours after admission): fluid turbid; 1,320 cells per c.mm., (lymphocytes 80 %); total protein 100 mgms. per 100 ml., excess of globulin; chlorides 750 mgms. per 100 ml; no bacteria (including tubercle bacilli) seen in films; culture sterile.

Subsequent Course. On September 26th (6th day) her white-cell count was 8,000 per c.mm., (polymorphs 44 %, eosinophils 3 %, basophils 2 %, lymphocytes 46 %, large mononuclears 5 %).

The patient was treated with 60,000 units of penicillin 3 hourly for five days, and sulphadiazine to a total of 34 g. over 4 days. Her temperature settled in 36 hours after admission and her general condition rapidly improved.

On September 29th (9th day) the left pupil was larger than the right, and there was left-sided ciliary injection. Neck rigidity had disappeared, and Kernig's sign was negative. Lumbar puncture: fluid slightly opalescent; 33 cells per c.mm.; total protein 40 mgms. per 100 ml., globulin not in



excess; no bacteria seen in films; culture sterile.

On October 6th (16th day) the pyrexia recurred and persisted for 48 hours. The tip of the spleen was just palpable. On October 8th (18th day) she insisted on taking her discharge from hospital because she was worried about her four children. She attended the medical out-patient department on October 20th (30th day) when she felt well but showed right-sided ciliary injection and bilateral blurring of the edges of the optic discs was seen. Between the 40th and 90th days of her illness the patient reported to the out-patient department on three occasions, complaining of severe headache. Eleven months after the illness began she still had headaches "behind the eyes" and stiffness of the neck in the morning.

The combination of acute aseptic meningitis and ciliary injection suggested a leptospiral infection (Buzzard and Wylie, 1947), and a diagnosis of *L. canicola* infection was confirmed by the serological findings which are reported below.

Until very shortly before the illness began the patient had a puppy which had diarrhoea and she frequently cleared up its urine and faeces. She had disposed of the puppy before coming to hospital and could not trace it.

### Case 3.

A woman, aged 40, was admitted to hospital on October 1st, 1947, with the following history.

On September 23 she had a headache, malaise and shivering. Next day there were aching pains in the limbs. The patient collapsed on getting out of bed and her temperature was found to be 105°F. On the 3rd day meningitis was suspected and a course of sulphathiazole treatment begun. On the 4th day vomiting became troublesome, and a mistiness of vision developed in both eyes and lasted for 48 hours. The patient was unable to see objects directly ahead but could see things "out of the corner of her eyes" fairly clearly. On the 5th day spots, similar in appearance and distribution to erythema nodosum, appeared on both legs, but they cleared in about 2 days. By the 8th day it was evident that the condition was not responding to sulphonamides and the patient was sent to hospital for further investigation.

On admission - ninth day of the illness. The patient still complained of severe occipital headache and pain at the back of her neck. There were now no pains in the limbs and no further nausea or vomiting. Micturition was normal, but she was severely constipated. She looked ill and was flushed and perspiring freely. Temperature 101°F., pulse-rate 86, respiration-rate 24 per min. Herpes labialis was present. There was no rash or lymphadenopathy. There was slight but definite neck rigidity, and the pain in her neck was much increased by flexing the cervical spine. Kernig's and Brudzinski's signs were both negative. No other abnormality in the central nervous system was noted. There was

neither conjunctivitis nor suffusion of the eyes. The optic fundi were normal, and rough tests showed no alteration in the visual fields. Blood-pressure was 120/80 mm. Hg. The cardiovascular system was normal apart from a soft basal systolic bruit. The urine was sterile on culture, contained a trace of albumin, and the centrifuge deposit showed a few red blood corpuscles and some leucocytes. Blood count: 3,700,000 red cells per c.mm., 6,000 white cells per c.mm. (polymorphs 69 %, stab cells 3.5 %, metamyelocytes 1.5 %, lymphocytes 19 %, monocytes 5 %, atypical lymphocytes 2 %), haemoglobin 11 g. per 100 ml. (76%). Lumbar puncture: fluid clear; initial pressure 120 mm water; 48 cells per c.mm.; protein 20 mg. per 100 ml; chlorides 710 mgms. per 100 ml; glucose 5.7 mgm. per 100 ml; culture sterile.

Subsequent Course. On October 3rd (10th day) her headache was still severe and she was perspiring freely; no change in physical signs. Lumbar puncture: 233 cells per c.mm. (lymphocytes 95 %, epithelial cells 5 %); protein 20 mgm. per 100 ml; chlorides 740 mg. per 100 ml; culture sterile. Blood urea 40.5 mgms. per 100 ml; Paul-Bunnell reaction negative.

Leptospirosis was considered as a diagnosis and the agglutination reaction was found to be positive for *L. canicola* and for *L. ictero-haemorrhagiae*, though to a lower titre. During the next few days the patient gradually improved, and by the fifteenth day her temperature was normal and the symptoms had disappeared.

On October 14th (21st day) the headache recurred, though less severely, and the temperature rose to 100.6°F. with early morning remissions. This relapse lasted for ten days, during which the symptoms improved and the temperature fell slowly. Recovery thereafter was uneventful and the patient was discharged from hospital on the 51st day. For the next fortnight the patient complained of weakness of the leg muscles, affecting particularly dorsiflexion of the feet. Her hair, which had begun to fall out while she was in hospital, was getting thinner, and the patient feared she might be going bald. When she was seen again 3 months later, however, the hair had started to grow again and the muscular weakness had cleared up.

For six months before her illness the patient had owned a dog which was in bad health. The detection of infection of this dog by *L. canicola* is described below.

#### Case 4. Archway Hospital.

A soldier, aged 18 years, was admitted to hospital on April 5th, 1948. During the 4 days before admission, he had suffered from listlessness, vomiting, and abdominal pain. For 3 days he had had increasingly severe headache.

On Admission. Fourth day of illness. Temperature 99.8°F, pulse-rate 72, respiration-rate 20 per min. There was slight right-sided ciliary injection. The cranial nerves and optic discs were normal. The right ear-drum was normal and the left showed an old scar. Neck rigidity was well-marked, and Kernig's sign was strongly positive. A clinical diagnosis of acute meningitis was made. Lumbar puncture (an hour after admission): fluid opalescent; pressure 130 mm. water; 380 cells per c.mm., all lymphocytes; total protein 400 mg. per 100 ml., excess globulin; chlorides 680 mg. per 100 ml; no bacteria (including tubercle bacilli) in stained films; culture sterile. Agglutination reaction negative to *L. ictero-haemorrhagiae* and *L. canicola*.

In view of the conjunction of lymphocytic meningitis and ciliary injection, leptospira infection was suspected. The patient was treated with sulphadiazine - a total of 22 g. in 2 days, and 100,000 units of penicillin 4 hourly for 4 days.

Subsequent Course. On April 7th (6th day) the patient was afebrile and free of all symptoms. There was no ciliary injection; neck rigidity had disappeared, and Kernig's sign was negative. The spleen was never palpable. Next day a blood sample was taken which gave a strong positive agglutination with *L. canicola* and an insignificant reaction with *L. ictero-haemorrhagiae*.

On April 12th (11th day) lumbar puncture: fluid opalescent; 364 white cells per c.mm., all lymphocytes; 14 red cells per c.mm.; total protein 60 mgms. per 100 ml; slight excess globulin; chlorides 700 mgms. per 100 ml; no bacteria or tubercle bacilli in stained films; culture sterile. On April 15th (14th day) the cerebro-spinal fluid contained 255 white cells and 1415 red cells per c.mm. Fluid was inoculated into two hamsters and into leptospira culture medium. Blood count on April 17th (16th day); Hb. 110 %; 8,200 white cells per c.mm. (polymorphs 46 %, eosinophils 1 %, lymphocytes 4.9%, large mononuclears 4%).

This patient could not remember any recent contact with dogs.

#### Serological Investigations.

Case 3 provided the most complete material, so the serological investigations dealing with it will be considered in detail although in the main they apply to all.

Case 3. A sample of serum taken on the 12th day of illness agglutinated *L. canicola* to a titre of 1/3,000 and *L. ictero-haemorrhagiae* in dilutions of 1/10 and 1/30 only. Further



specimens tested during the illness and after recovery showed the characteristic rise and maintenance of agglutinins against the homologous leptospira, and the fall and disappearance of the co-agglutinins.

Samples of the second specimen of serum (titre 1/10,000; 1/300) were absorbed with heavy suspensions of *L. canicola* and *L. icter-haemorrhagiae* respectively, and the residual agglutination titres estimated. As is shown in Table III, *L. canicola* removed all the agglutinins, whereas *L. ictero-haemorrhagiae* absorbed its homologous agglutinins but only reduced the titre against *L. canicola* to 1/3,000.

Protection tests were carried out to confirm these results. Two hamsters (*Cricetus auratus*) were given 1.0 ml. of the patient's serum intraperitoneally, and, along with two untreated control hamsters, they were inoculated with 0.5 ml. of culture of a virulent strain of *L. canicola*. In the same way, two guinea-pigs received 2.0 ml. of serum, and, with two controls, 0.5 ml. of culture of a virulent strain of *L. ictero-haemorrhagiae*. All the guinea-pigs died. The control hamsters died but the two survived which had received the patient's serum, showing that the serum contained protective antibodies against *L. canicola* only.

Case 1. On the 24th day after onset this patient's serum agglutinated *L. ictero-haemorrhagiae* to a titre of 1/1,000, but tests with *L. canicola* were not carried out. On the 50th day the serum was positive to *L. canicola* in dilutions up to 1/3,000 and to *L. ictero-haemorrhagiae* to 1/100 only. Absorption tests proved this also to be a case of canicola fever. On approximately the 100th day the titres were *L. canicola* 1/300; *L. ictero-haemorrhagiae* 1/10.



Case 2. Serum from this patient was first examined on the 30th day of disease. Agglutination was positive with *L. canicola* to a titre of  $1/3,000$  and with *L. ictero-haemorrhagiae* to  $1/300$  with a trace at  $1/1,000$ . With the latter organism, however, agglutination was only partial in all dilutions, and it presented a picture quite different from the typical reaction of which occurred with *L. canicola*. Absorption tests confirmed that the infection was due to *L. canicola*. I tested her serum eleven months after the illness began and it agglutinated both *L. canicola* and *L. ictero-haemorrhagiae* to an end-point of  $1/100$ .

Case 4. Cerebro-spinal fluid taken on the 4th day was negative against both species of leptospiras. A sample of blood of the 7th day agglutinated *L. canicola* in dilution of  $1/3,000$  and *L. ictero-haemorrhagiae* to  $1/10$  with a trace at  $1/30$ . This is an unusually high reading for the 7th day. The onset, however, was insidious and this may not be an accurate date. A second specimen of cerebro-spinal fluid of the 14th day agglutinated *L. canicola* in dilutions of  $1/10$  with traces at  $1/30$ . The fluid was inoculated into hamsters and into leptospira medium but the strain was not recovered. This is not surprising in view of the treatment with penicillin which is very active against *L. canicola*.

In cases 1, 2 and 4 I was responsible for the agglutination tests against *L. ictero-haemorrhagiae* and in cases 2 and 4 for the pathological examinations of cerebro-spinal fluid and blood.

Dr. Broom carried out the tests against *L. canicola*, except the last specimen from Case 2 which I tested with this species.

It has been recorded in the clinical reports above that three of the four patients had a dog when the illness began. The

dog of patient No. 3 had been ailing for some months. Dr. K. S. Rodan sent a specimen of blood from it for examination, and this serum agglutinated *L. canicola* to a titre of  $1/3,000$  and *L. ictero-haemorrhagiae* to  $1/30$ . The dog was destroyed before any other investigations could be made, but there seems little doubt that it was the source of the patient's infection. Patient No. 2 had often cleaned up urine and faeces from her dog, which had diarrhoea, but she disposed of the animal just before her illness began and could not trace it. The dog of patient No. 1 appeared to be quite well but was accidentally killed after the patient's illness began; Senthille et al. (1946) reported infection of three people from dogs which, though apparently healthy, were found to be excreting *L. canicola* in the urine.

These four infections by *L. canicola* and the only other one previously identified in this country (Baber and Stuart, 1946) show the disease in the meningeal form. In countries where the infection is better known meningitis is not always found. It can be expected that both forms of the infection will be detected in this country in future.

A variety of symptoms and signs is notable in reports of canicola fever, whether meningitis is present or not, as is shown by a summary of symptoms in our four patients. The onset included headache and fever in all, with vertigo or vomiting or abdominal pain. During the illness, eye signs developed in all—conjunctival or ciliary injection, photophobia, inequality of pupils, mistiness of vision, and blurring of edge of retinal disc. The relative prominence of such eye signs has been noticed in the meningeal forms of Weil's disease (Buzzard and Wylie, 1947); optic neuritis or perineuritis has been commented on in Weil's disease by Venco (1933) and others. Stiff neck was present in all and Kernig's sign was present in three; a muscular weakness (of dorsiflexion of the feet) was present in only one. One patient had pains in the limbs. Three had morbilliform rash, in the form of small red spots or larger macules like erythema

nodosum. In one of these three herpes labialis was also present. The spleen was just palpable in one case; thinning of the hair was noted in another. Features recorded by others which our patients did not show include diarrhoea, pulmonary congestion, epistaxis and mild nephritis.

In the cerebro-spinal fluid the maximum abnormal findings varied in our patients from 290 to 1,320 cells per c.mm., (almost all lymphocytes), 20 - 400 mgms. of protein per 100 ml. In three, leucocyte counts showed little abnormality except a relative lymphocytosis in one.

One of our patients had recurrent headaches up to 90 days, and another had a relapse with headache and fever at the 21st day; such relapses have been noted before in canicola infections. Jaundice or other evidence of liver damage is unusual and when it does occur is mild. Death from the disease is known in only one patient (Van Thiel, 1948, p.139).

In any form of the infection clinical diagnosis is difficult. In the presence of a severe conjunctivitis and a rash, if secondary syphilis, the exanthemata, and certain skin diseases have been excluded, leptospirosis should be considered. Jaundice is unfortunately thought to be an essential clinical feature, whereas even with *L. ictero-haemorrhagiae* it may be absent or transient. Should there be also an "aseptic" meningitis, the presumptive diagnosis is still more strengthened. It is interesting to observe that the swineherds' disease (*maladie des porchers*), an endemic and occupational disease of Savoy, characterised by diarrhoea, rash, haemoptysis, and sometimes a lymphocytic meningitis, and long believed to be due to a virus,

was shown by Gsell (1944) to be a leptospiral infection. From another aspect of diagnosis, in any example of lymphocytic meningitis one type or another of leptospiral infection should be suspected.

The final diagnosis of canicola infection depends on agglutination tests of the patient's blood and, if possible, isolation of the leptospira from the blood or cerebro-spinal fluid by means of hamsters.

Dr. Broom allows me to state that since the paper by Laurent et al., was published he has found five more human infections by *L. canicola* in England. Four of these were active infections and the other was a past infection which happened probably several years ago but was still detectable by agglutination test of the serum. In this instance, the patient attributed recurrent neurological symptoms to the infection.

Clinical signs and the history of the illness cannot yet provide the basis of diagnosis. This is shown by a group of patients whom I observed in London recently. Four children had spent a day at the home of one of them where there was a dog. A week later, the dog became ill and on the same day the children all began a mild illness with meningitis, which was confirmed by an increase of cells in the cerebro-spinal fluid. The circumstances suggested leptospirosis *canicola*, but agglutination tests at the 8th and 25th days with *L. ictero-haemorrhagiae* and *L. canicola* did not give a result with the serum of any of the children. I inoculated alkaline urine into young mice and young guinea-pigs and Dr. Broom injected it into hamsters, but with no results.



By contrast on September 7, 1948, I tested a serum which clearly revealed another active infection by *L. canicola*.

The patient, W.F., is a man aged 55 years. At the beginning of August, 1948 he had abdominal pain and vomiting for three days. He was well for the next two weeks and on 19th August a febrile illness began with temperature of 99 to 102°F. and a rigor. This continued for 8 days, accompanied by injection of the throat and enlargement of some superficial lymph glands. On 27th August he was admitted to hospital with headache, neck rigidity and marked conjunctivitis. Cerebro-spinal fluid contained 450 leucocytes per c.mm., 50 per cent being polymorphs. He recovered within a few days, but had frontal headache in the mornings for a week. I found that his serum, taken on 7th September, agglutinated *L. canicola* to an end-point of 1 in 300,000 and *L. ictero-haemorrhagiae* to 1 in 1,000.

This patient said that he has been frequently in contact with a friend's dog and often allows the animal to lick his face. The dog has been ill for two or three months with much loss of weight and of hair. I am trying to get the dog to test its serum for agglutinins and to try to isolate *L. canicola* from its urine.

The total of human infections by *L. canicola* recognised in the British Isles is, therefore, twelve, - ten found in the acute stage and two after recovery.

-----oOo-----

The Summary (pages 178 - 182) follows the References.

Occupation etc. Related to 983 Cases of Weil's Disease known to me in the British Isles from July, 1933 to July, 1948 (inclusive).

Occupation	Number	Per Cent of Total
Fish-worker	216	22.1
Other food-handler incl. Butcher, Slaughterer, Fish-monger, Tripe-scraper.	21	2.1
Coal-miner	139	14.2
Sewer-worker	79	8.1
Army, incl. A.T.S.(2), P.O.W. (6)	67	6.9
Navy (British, Free French)	19	1.9
Royal Air Force	11	1.1
Bathing or Paddling incl. Sudden Immersion(7)	48	4.9
Farm-worker	45	4.6
Worker in water incl. Canal, Gravel-pit, Water-cress, River Drainage	16	1.6
Bite or scratch by rat, dog or ferret	11	1.1
Builder, etc.	9	0.9
Gardener, etc.	4	0.4
Laboratory worker	2	0.2
Miscellaneous incl. rag-work, bottle-washing	29	3.0
Unrecorded or not yet analysed	267	26.9
TOTAL	983	100.0

This table has been compiled from some large groups of cases and small groups or individual cases, as follows:-  
Large Groups.

- |  |           |
|--|-----------|
| 1. Alston, J.M. 3rd Internat. Cong. Microbiol. 1939                                  | 274 cases |
| 2. Broom, J.C. and Alston, J.M. (1948)   | 195 "     |
| 3. Smith J. (personal communication)   | 100 "     |
| 4. Gardner and Wylie (1946)  | 182 "     |
| 5. Stuart, R.D., various publications and personal communications not included in 1. | 82 "      |

Small Groups and single cases, many additional to above published or communicated privately by:-

- |                         |                         |
|-------------------------|-------------------------|
| Alston, J.M. (1935)     | Maxwell, J. (1936)      |
| Bedson, S.P. (1934)     | Middleton, J.C. (1938)  |
| Carragher, A.E. (1945)  | Neale, A.V. (1935)      |
| Coles, W.E.K. (1936)    | Rees, W.E. (1939)       |
| Cross, R.M. (1945)      | Robertson, K.M. (1946)  |
| Davidson, L.S.P. (1939) | Sladden, A.F. (1939)    |
| Evans, J.A. (1946)      | Stewart, A. (1944)      |
| Faulds, S.J. (1934)     | Stuart, R.D. (1939)     |
| Gauld, W.R. (1947)      | Stuart, R.D. (1939b)    |
| Halstead, E.A.M. (1935) | Swan, W.G. (1938)       |
| Hart, V.L. (1944)       | Tulloch, W.J. (1946)    |
| Jackson, H. (1946)      | Watson, G.W. (1935)     |
| Jenkins, T.H. (1946)    | Wigmore, J.B.A. (1936)  |
| Lendrum, J.D. (1936)    | Willcox, A. (1944)      |
| Mackie, T.J. (1946)     | Wolstencroft, J. (1935) |

|| N.B. In this list, space has allowed only the first name ||  
 || to be given when there was more than one author. ||

SELECTED SURVEYS OF *L. ICTERO-HAEMORRHAGIAE* IN RODENTS.

TABLE 2.

Date of Publication	Author	Country	Species of Host	Percentage positive
1917	Ido, Hoki et al	Japan	<i>Mus decumanus</i>	40.0
1917	" " "	"	<i>Mus alexandrinus</i>	0.8
1922	Stevenson	England	Rats	30.0
1927	Buchanan	Scotland	<i>Rattus norvegicus</i>	37.0
1927	"	"	Field mouse	
1931	Elton	England	Field mice	8.0
1934	Schüffner	Holland	Rats	28.0
1935	Anderson (unpublished)	Bombay	<i>R. norvegicus</i>	32.0
		India	<i>R. rattus</i>	0
			<i>Gunnys varius</i>	7.0
1935	Alston	London	<i>R. norvegicus</i> from sewers	
1936	Malmgren	Sweden	Rats	52.0
1937	Serjito et al.	E. Indies	<i>R. brevicaudatus</i>	50.0
1937	Korthof	E. Indies	White rats	
1938	Mason	Liverpool	Rats from sewers	32.0
		England	Rats from house	15.0
			Rats from docks	9.0
1938	Kirk	Egypt	<i>R. rattus</i> , <i>R. norvegicus</i>	0.
			<i>Arvicanthus testicularis</i>	0.
1938	Sawers	Queensland	<i>R. rattus</i>	1.0
			<i>R. culmorum</i>	8.0
1938	Roelcke	Germany	White rats	13.0
1938	Smith	Aberdeen	Rats	27.0
1939	Thjotta et al.	Norway	Rats	41.0
1939	Meyer, et al.	California	Rats	
1942	Savino et al.	Argentina	Rats	
1943	Larson	U. S. A.	Rats	
1944	Petersen	Denmark	<i>R. norvegicus</i>	85 in older animals

TABLE 2.

In some instances, the species of *Leptospira* was not fully identified as *L. ictero-haemorrhagiae*.

SELECTED REPORTS OF I. ICTERO-HAEMORRHAGIAE IN ANIMALS OTHER THAN RODENTS.

TABLE 3.

Dates of Publication	Author	Country	Species of host	Percentage positive
1924 - 25	Dunkin et al.	England	Wild fox 1	
1935	Smith J. (unpublished)	Scotland	Silver foxes (3)	
1935	Sander	Germany	Pig (uncertain)	
1936	Uhlenhuth et al.	Germany	Dogs,	6
1937	Petersen et al.	Denmark	Dogs	35
1938	Klarenbeek	Holland	Dogs (57)	
1939	Van der Walle	Belgium	Dogs (14)	
1941	Larson	U. S. A.	Dogs	
1943	Kathe	Czecho-Slovakia	Horses (2)	
1943	Kathe	Czecho-Slovakia	Cattle (2)	
1946	Stuart	Scotland	House dogs	6
1946	Stuart	Scotland	Kennel dogs	28
1947	Roch et al.	Central Europe	Pigs	

TABLE 3.



TABLE 4.

## DISTRIBUTION BY AGE OF 100 CASES OF WEIL'S DISEASE IN BRITISH ISLES 1940 - 1946.

From Broom, J.C. and Alston, J.M. (1948).

	0 - 15 years	16 - 30 yrs	31 - 45 yrs	46 - 60 yrs	Over 60 yrs	Total	Age unknown
12		31	31	21	5	100	95

Distribution of  
Cases by Age

TABLE 4.

DISTRIBUTION BY MONTHS OF 50 CASES OF WEIL'S DISEASE IN LONDON SEVEREIN 1934 - 1946 MOSTLY FROM  
RECORDS OF MY OWN INVESTIGATIONS (J.M. ALSTON).

Year	MONTH OF YEAR												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1934					1		2			1	2		6
1935				2	1			2	1	1			7
1936							2	2					4
1937						1	2	1	2				6
1938						1	1	1	1		3	2	9
1939	1	1		1	1								5
1940									1				1
1941										1	1		2
1942									2				2
1943	2		1						1				4
1944			2										2
1945			1						1		1		3
1946													
1934-46	3	1	4	3	3	3	7	6	8	3	7	2	50

The three months (in all years) Dec. Jan. & Feb. showed 6 cases = 12 per cent.  
The three months (in all years) Sept. Oct. & Nov. showed 18 cases = 36 per cent.

TABLE 6.

DISTRIBUTION BY AGE OF CASES OF WEIL'S DISEASE ENDING IN DEATH OR RECOVERY  
IN THE BRITISH ISLES, 1940 - 1946.

0 - 15 years.	16 - 30 yrs.	31 - 45 yrs.	46 - 60 yrs.	Over 60 years	Total	Age unknown
2	4	4	6	3	19	6
10	19	22	15	2	68	21

Distribution of  
deaths by age.

Distribution of  
recoveries by age.

Age 0 -45 years: Case fatality rate = 16 per cent.

Age 46 - over 60 years: Case fatality rate = 42 per cent.

TABLE 6.

HIGHEST RECORDED UREA CONCENTRATION (MG. UREA PER 100 C.C. OF BLOOD) IN FATAL AND RECOVERED CASES OF WEIL'S DISEASE.

Constructed by J.M. Alston from material collected for "Weil's Disease" (Broom and Alston, 1948) and from his own unpublished records.

9 Fatal Cases	22 Recovered Cases		
153	33	96	284
234	52	108	317
245	53	145	321
340	53	164	328
480 320	62	170	355
552	63	250	366
701	63	265	
750	67	273	
Mean: 419	Mean: 177		
Range: 153 to 750	Range: 33 to 366		

Records of more than one test in single cases.

Fatal Case: at 15th day of illness 546

18th " " " 701 (day of death)

Recovered Cases 1. At 6th day of illness 125  
 " 8th " " " 145  
 " 13th " " " 46  
 " 21st " " " 32

Recovered Cases 2. At 10th day of illness 355  
 " 15th " " " 380  
 " 21st " " " 84  
 " 28th " " " 140  
 " 53rd " " " 72

Recovered Cases 3. At 7th day of illness 103  
 " 10th " " " 220  
 " 14th " " " 265  
 " 15th " " " 317  
 " 18th " " " 278  
 " 22nd " " " 175  
 " 24th " " " 90



## WEIL'S DISEASE IN 40 LONDON SEWER-WORKERS 1934 - 1948.

Investigated bacteriologically by J.M. Alston.

No.	Month	Age	Type of Work	Time in Sewer Work	Examn. for L. I. H.	Highest Adhesion or Aggltn 1.	Highest Leucocyte Count	Outcome
1.	1934 June	52	F.	5 yr.	B. 20d - U. 13d + U. 17d -	St. pos Ad	15,400	R.
2.	1934 July	35	F.	9 m.		160 Ad	17,000	R.
3.	1934 Nov.	52	B.	6 m.		160 Ad	25,000	D.
4.	1935 Apr.	47	B.		U. 18d -	1000	4,800	R.
5.	1935 Apr.	33	F.		B. 5d + B. 11d -	Pos. Ad	23,900	D.
6.	1936 July	43	?		U. - B. -	3,000		R.
7.	1936 Aug.	66	B.	Occas.		30,000	11,600	R.
8.	1936 Aug.	59	Rat catcher		Stained in P.M. tissues	100		D.
9.	1937 June	54	B.			3,000		R.
10.	1937 July					300		D.
11.	1937 July	52	F.		B. 24d - U. 32, 34, 40d -	3,000	9,000	R.
12.	1937 Sep.	45	?		B. 9d - U. 9d -	3,000	14,000	R.
13.	1938 June	31	B.	5 y.	B. 6d + B. 9d -	1,000	24,200	R.
14.	1938 July	34	F.		B +	1,000		R.
15.	1938 Aug.	36	?		B. 7d -	3,000	12,500	R.
16.	1938 Sep.		F.	4 y.	B. +	Pos		R.
17.	1938 Nov.	42	F.	11m.	B. 5d +	1,000		R.
18.	1938 Nov.	56	F.		B. 6d - U. 6d -	10,000		R.
19.	1938 Dec.	24	F.	2 y.		30,000		R.
20.	1939 Feb.	43	B.		U. 12d -	10,000		R.
21.	1939 Apr.	61	?		U. - B. -	100,000		R.

CONTINUED

WELL'S DISEASE IN 40 LONDON SEWER-WORKERS 1934 - 1948.  
Investigated bacteriologically by J.M. Alston.

No.	Month and Year	Age	Type of Work	Time in Sewer Work	Examn. for L. I. H.	Highest Adhesion or aggltn. 1	Highest Leucocyte Count	Outcome
22	1939 May	?	?			300		R.
23	1939 June	38	F.			1,000		D.
24	1940 Oct.	36	F.		B. 4d +	300		D.
25	1941 Sep.	43	B.			1,000		R.
26	1941 Nov.	49	F.	3 M		1,000		R.
27	1942 Sep.	44	F.	3 M		300		R.
28	1942 Sep.	45	F.			1,000		R.
29	1943 Jan.	46	F.			1,000		
30	1943 Jan.	48	F.	7 M		300		R.
31	1943 Mar.	47	F				9,400	D.
32	1943 Sep.	49	?			1,000		R.
33	1944 Mar.	54	F	17Y		1,000		D.
34	1944 Mar.	34	F			1,000		R.
35	1945 Mar	45	B	Sev. yrs		10,000		?
36	1945 Aug.	59	F			1,000		R.
37	1947 Sep.	67	?			1,000		R.
38	1947 Oct.	36	?			3,000		R.
39	1947 Oct.	33	F	8Y +		3,000	10,400	R.
40	1948 July	36	F	10M	B. 5d+	3,000	20,400	R.

In "Type of Work" column, F. = Flusher, B. = Builder.

In "Outcome" column, R. = recovered, D = Died.

In "Examn. for L. I. H." column, B. = Blood, U = Urine; 4d, etc = 4th day, etc.

TABLE 9.

WEIL'S DISEASE IN 18 LONDON SEWER-WORKERS 1933 - 1948.  
 Investigated bacteriologically by H.C. Broom, J.C. Brown or S.P.  
 Bedson.

No.	Month and Year	Age	Type of Work	Time in Sewer Work	Examn. for L. I. H.	Highest Adhesion or aggltn.1	Highest Leucocyte Count	Out-come
1.	1933 Nov.		F.	1½ Y		400 Ad		R.
2.	1934 May	25	F.	3Wks	B.7d +	1,000	18,000	D.
3.	1934 Oct.	29	B	5Mth	B.9d - U.23d+	S. pos. Ad.		R.
4.	1934 Nov.	45	B	20Y		200 Ad		R.
5.	1935 May	44	F	8 Y	B.11d - U.16d+	1,000	6,600	R.
6.	1935 Aug.		B			Pos.		D.
7.	1935 Aug.		B			Pos.		D.
8.	1935 Oct.		B			Pos.		R.
9.	1935 Oct.		?			3,000		R.
10.	1935 ?		B			Pos.		R.
11.	1936 July		?			Pos.		R.
12.	1936 July		?			Pos.		R.
13.	1937 Aug.	28	F	15 Y.		10,000		R.
14.	1937 Sep.	59	F			100		D.
15.	1938 March					3,000		R.
16.	1938 Nov.	29	B			3,000		R.
17.	1938 Dec.	62	?			300		R.
18.	1939 Jan.	32	B			1,000		R.

In "Type of Work" column, F. = Flusher, B. = Builder.

In "Outcome" column, R. = recovered, D. = Died.

In "Examn. for L. I. H." column, B. = blood, U. = urine;  
 4d etc. = 4th day, etc.

TABLE 10.

DISTRIBUTION BY AGE OF 46 CASES OF WEIL'S DISEASE IN LONDON SEWERMEN, 1933 - 1948.

Under 30 years.	30 - 39 yrs.	40 - 49 yrs.	50 - 59 yrs.	60 years and over.	TOTAL.
5	12	16	9	4	46

Distribution of  
Cases by Age.

TABLE 10.



TABLE 11.

Source of Serum	Agglutination Titre	Fate of two guinea-pigs treated with Serum 1 hour before injection of <i>L. ictero-haemorrhagiae</i>	
		0.5 c.cm. Serum	1.5 c.cm Serum
K. (Sewerman)	1 in 100	1 survived, 1 died after 9 days	2 survived
Pe (Sewerman)	1 in 100	2 survived	2 survived
Pr. (Sewerman)	1 in 100	2 survived	2 survived
E. (Sewerman)	1 in 100	2 survived	1 survived, 1 died after 15 days
Al. (Control)	Negative	2 died after 6-7 days	2 died after 6-7 days
Au. (Control)	Negative	2 died after 6-7 days	1 survived, 1 died after 6-7 days

All animals which died were examined thoroughly post-mortem and the typical signs of haemorrhagic jaundice were discovered. *Leptospira* were sought in one animal of each pair and found.

It is considered that this experiment demonstrates that protective antibodies accompany agglutinins in the serum of certain sewer-men.

TABLE 11.

TABLE 12.

TABLE SHOWING RESULTS OF AGGLUTINATION TESTS ON RABBITS.

		1/10	1/30	1/100	1/300	1/1,000
R. 201	Before	+	-	-	-	-
	5 mins	+	+	+	-	-
	7 hrs	+	+	+	-	-
	24 "	+	+	+	-	-
	48 "	+	+	+	-	-
	120 "	+	-	-	-	-
R. 202	Before	-	-	-	-	-
	5 mins	+	+	+	-	-
	7 hrs	+	+	+	±	-
	24 "	+	+	+	±	-
	48 "	+	+	+	±	-
	120	±	-	-	-	-
R. 262	Before	+	-	-	-	-
	5 mins	++	+	+	±	-
	6½ hrs	+	+	+	-	-
	24 "	+	+	-	-	-
	48 "	+	-	-	-	-
R. 263	Before	-	-	-	-	-
	5 mins	++	+	+	±	-
	6½ hrs	++	+	+	-	-
	24 "	+	+	+	-	-
	48	+	-	-	-	-

CASE-FATALITY RATES RECORDED IN DIFFERENT COUNTRIES ALONG WITH PERCENTAGE OF JAUNDICE.

Dates.	Author.	Country.	Percentage of Jaundice.	No. of Cases.	Case Fatality Rate.
1922	Inada, 1922.	Japan	High		30 per cent
1933-37	Alston, et al '37	British Isles	100 (approx.)	142	15 per cent
1934-43	Petersen 1944(a)	Denmark	100	59	25-30 per cent
1937-43	Stuart (unpub.)	Scotland	83	59	25 per cent
1924-36	Esseveld 1937	Holland	--	756	10 per cent
1934-43	Petersen 1944(a)	Denmark	35	177	10 per cent
1934-38	Davidson et al.	N.E. Scotland	60	104	6 per cent
1923-32	Erber 1934	France	--	286	6 per cent
1916-17	Dawson et al 1917	Belgium British Army	50	-	5 per cent

DISTRIBUTION BY MONTHS OF 195 CASES OF WEIL'S DISEASE IN GREAT BRITAIN 1940 - 1946.  
 TABLE CONSTRUCTED FOR THIS THESIS BY J.M. ALSTON FROM MATERIAL USED IN PAPER ENTITLED  
 "WEIL'S DISEASE" BY BROOM & ALSTON (1948).

Year	Month of Year.												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1940	2	2	3	2	3	5	-	1	1	3	3	-	25
1941	-	1	-	1	-	2	4	3	9	4	5	2	31
1942	1	-	-	4	2	-	1	1	4	4	3	1	21
1943	7	3	1	1	1	2	3	3	4	6	5	-	36
1944	2	1	2	1	2	2	1	3	7	3	4	-	28
1945	1	3	3	-	1	1	1	3	3	2	3	-	21
1946	2	3	2	1	-	2	-	4	3	11	5	-	33
1940-46	15	13	11	10	9	14	10	18	31	33	28	3	195



TABLE RECORDING TITRE OF AGGLUTINATION AT KNOWN DAY OF ILLNESS IN 22 PATIENTS.

1	2	3	4	5	6	7	8	9	10	11- 15	16- 20	21- 25	26- 30	31- 40	41- 50	51- 60	61- 70
									1T								
						-				3H		3T					
												3T					
										3H	3T	3T	30T	10T			1T
	-				1T					1H		3T					
						1T				10T							
									1T			30T					
						10T										10T	
			-			3T						100T					
			10	3H													
					30			1T		3H							
						3T											
					-							1T		3T			
										3H							
										3T							

Titre indicated thus: - negative

10, 30 = 1/10, 1/30

1H, 3H = 1/100, 1/300.

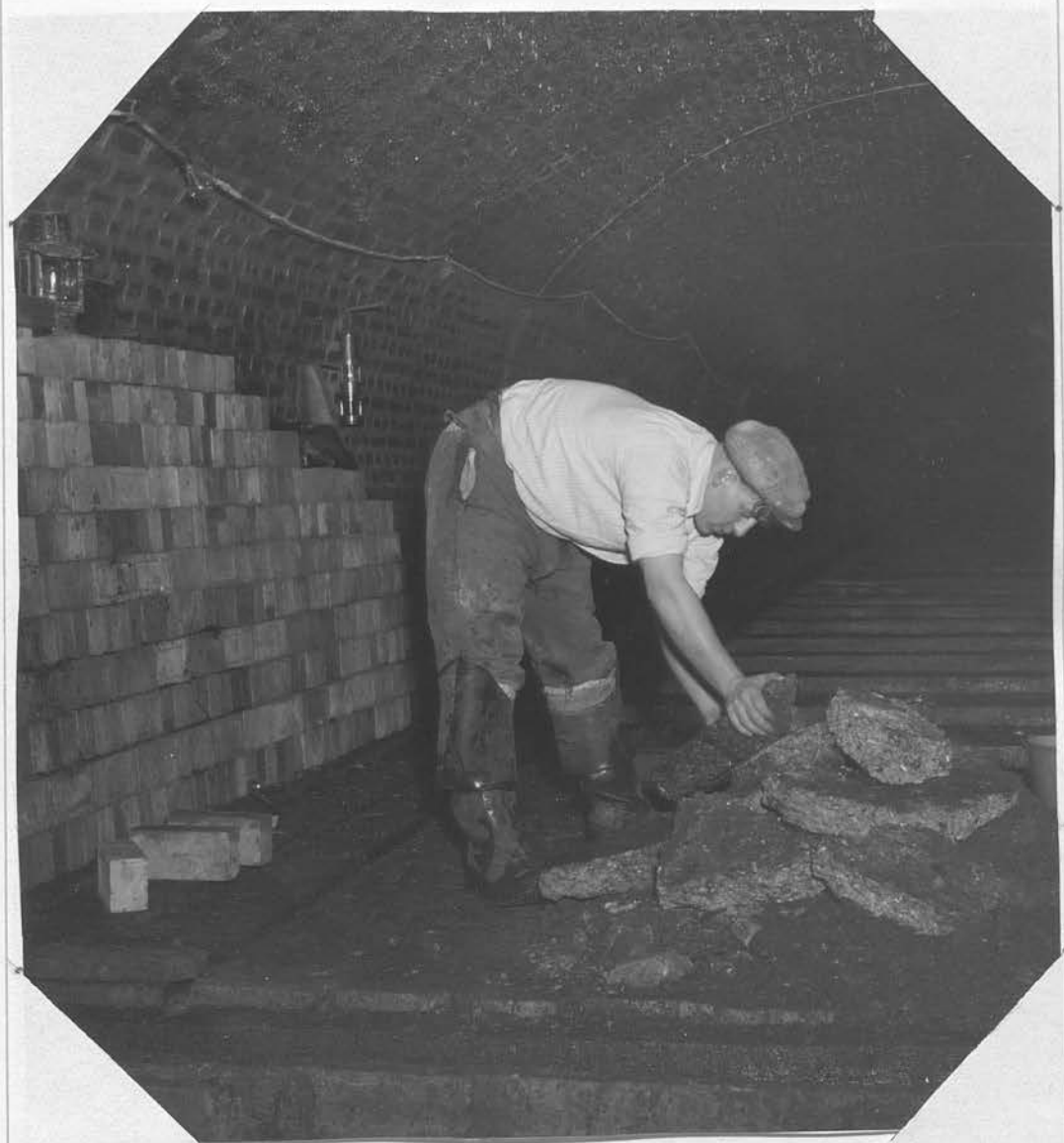
1T etc. = 1/1,000, etc.

RECORDS OF ACTIVE INFECTIONS BY L. CANICOLA IN MAN.

Country	Date of First Record	First and Some Later Writers	Total for Country
Holland	1934	Dhont et al. 1934 Schüffner 1941 Minkenhof 1948 Ruys (personal com.) 1948	52
Denmark	1938	Brammer et al. 1938 Petersen 1944 (a)	47
Germany	1938	Stödter (1938) same Tetzner 1938) patient	1
United States	1938	Meyer et al. 1938 Bruno et al. 1943 Tievsky et al. 1944 Rosenbaum 1946	7
China	1940	Snapper et al. 1940	2
France	1945	Kolochine-Erber 1945 Gaillemin 1946	7
British Isles	1946	Baber and Stuart 1946 Laurent et al. 1948 J.C. Broom (unpubl.) J.M. Alston ( " )	10
Norway	1946	Aalvik 1946 Grelland 1946	2
Porto Rico	1946	Cadillo et al. 1946	1
Switzerland	1946	Gsell 1946	9
Argentina	1947	Del Sel et al. 1947	7
			145

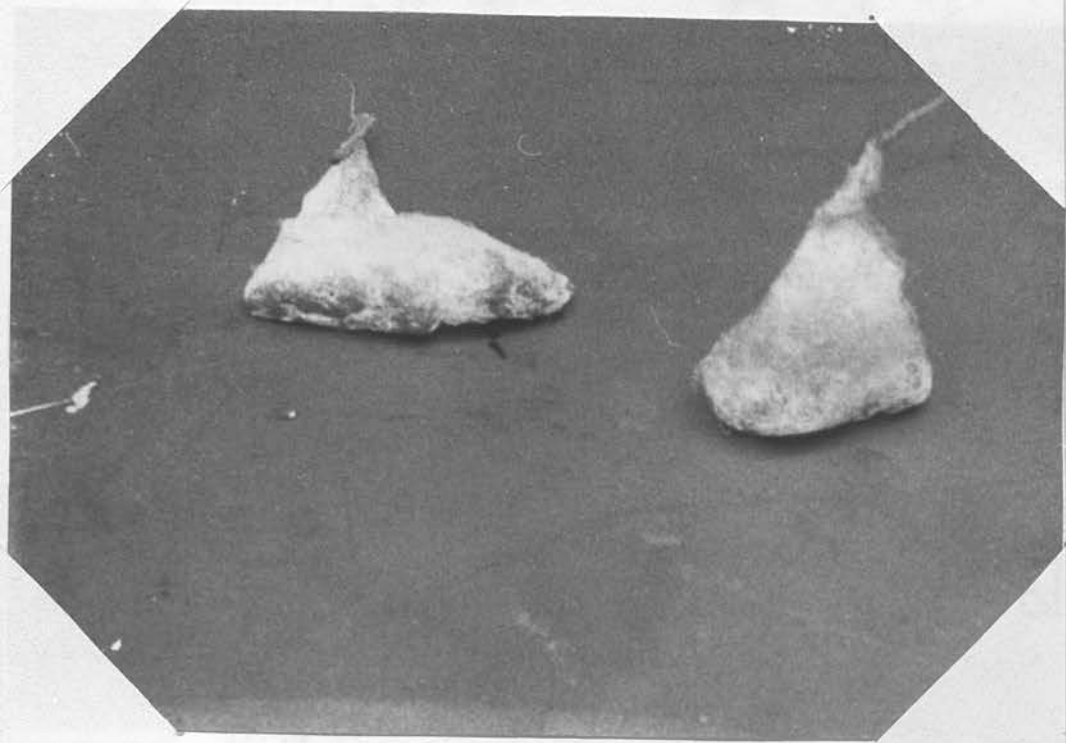


A London sewer below Chesham Place, S.W. taken in February, 1937. Builder's labourers are breaking and handling mud covered cement of floor of sewer.

PHOTOGRAPH II.

A London sewer, shewing a builder's labourer handling mud-covered cement work and showing bricks to be used to rebuild the floor.

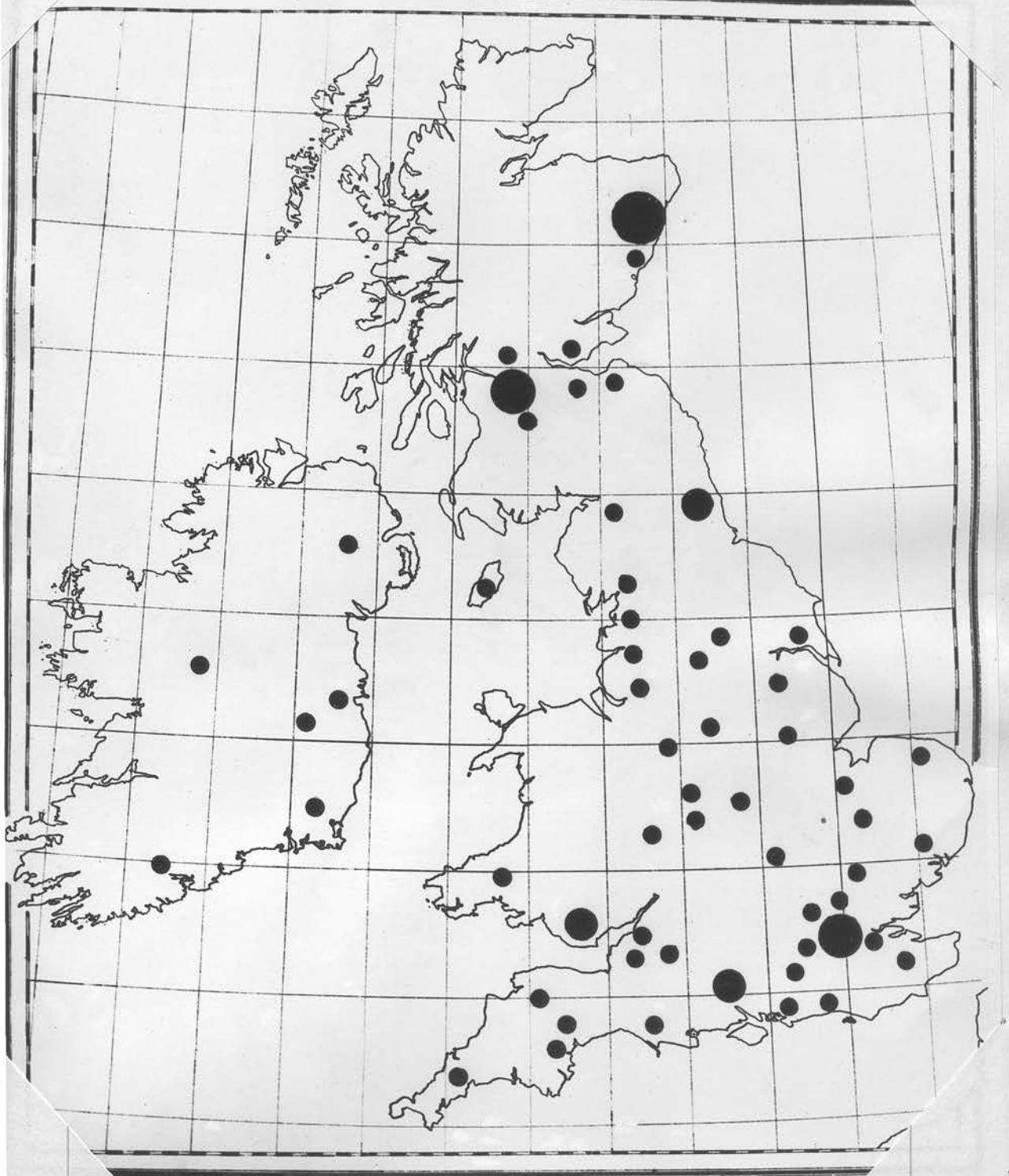


PHOTOGRAPH IV.

Rats faeces covered with fungus; another indication of rats presence. Rats are seen very little during the day by workers in the sewers.

MAP I.

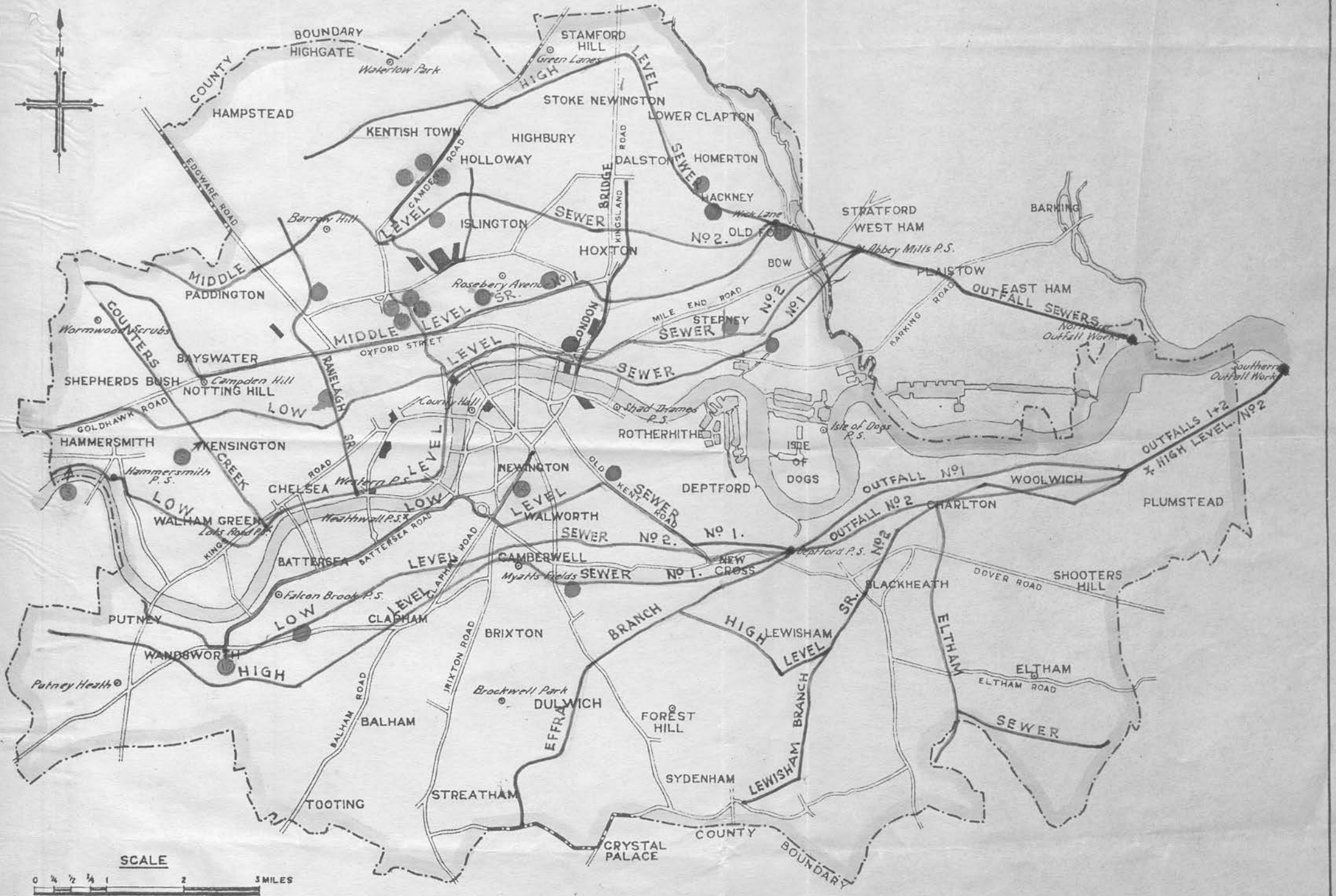
## BRITISH ISLES



Approximate Distribution of Cases in British Isles 1934-1948.

- indicates Aberdeen - 200 cases.
- " Glasgow and London - 100 cases each.
- " Newcastle, South Wales, Hampshire-50 cases each.
- " areas reporting one to ten cases each.

MAP II.



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SUMMARY.PART I. Leptospirosis Ictero-haemorrhagiae.

Weil's disease was defined as a clinical entity in 1886 and Japanese workers discovered in 1915 the spirochaete which causes it. H. Noguchi studied this organism carefully and named it *Leptospira ictero-haemorrhagiae*. The Japanese laid a firm and wide basis of knowledge of the infection and found that it is transmitted to men by rats and other rodents. From surveys in all parts of the world it was seen that rats in nearly all countries harbour *L. ictero-haemorrhagiae* and are the main source of infection in man.

I know of almost 1,000 instances of Weil's disease in the British Isles. The disease is strongly occupational in fish workers, coal miners, sewer workers, people bathing in fresh water and among people in other circumstances where rats are frequent. Details are given of investigation of the disease in some of the more frequent circumstances in the British Isles.

Dogs are infected and occasionally transmit the disease to man. Foxes and pigs are less often infected and some other animals more rarely still.

*L. ictero-haemorrhagiae* quickly dies in an acid medium, in strong sunlight and in salt water, and these facts can be related to the occurrence or absence of the infection in some situations.

Infection in man is usually by the contact of abraded or sodden skin with infected mud or water, but it may be by inhalation of water and by bites of rats, dogs or ferrets. Infection may be by ingestion through the mouth or by way of the conjunctiva.

Men are more often exposed to infection than women, but the sexes are equally susceptible. The disease may occur at any age and is more serious in older people.

There is a preponderance of Weil's disease in the autumn and early winter in most circumstances of infection except in bathing.

The incubation time is usually between seven and thirteen days.

The fatality rate depends on the number of mild anicteric or meningitic infections included.

Meningitis is the most prominent feature of many mild examples of the disease. This and other anicteric illnesses are not fatal.

*L. ictero-haemorrhagiae* is one of the most virulent species of leptospiros. It is clearly related to *L. canicola* and some other species but has no obvious antigenic relation to most. There is no sound evidence that the avirulent, free-living *L. biflexa* can become virulent. *L. ictero-haemorrhagiae* occurs in two antigenic forms - complete and incomplete.

Specific antiserum can protect animals from infection in some circumstances. The same is true of penicillin and streptomycin to which, in vitro, *L. ictero-haemorrhagiae* is very susceptible. These agents are probably effective in human patients if they are given in the first few days of illness.

My own experience of Weil's disease is of 79 instances in sewer-men and others. The system of sewers in London (as elsewhere) is greatly infested with rats which reach the sewers by broken drain-pipes. I have isolated *L. ictero-haemorrhagiae*

from rats from the <sup>we</sup> ~~sers~~ and from slime from the floor or lower walls of sewers. I found that the organism dies out rapidly in infected water and is quickly killed by ultra-violet light. I used culture of patient's blood, transfer of infection from blood and urine to guinea-pigs and agglutination tests of serum for diagnosis. I have analysed records of patients who were sewer-men as to seasonal incidence, age, type of work, length of time the men had been engaged at sewer and the case mortality, the period of the illness at which *L. ictero-haemorrhagiae* was obtained from blood or urine, leucocyte count, anaemia and blood urea. Among those who were not sewer-men, occupation was analysed. For both groups, the time and degree of production of agglutinins was studied.

I found that 20 per cent of 45 sewer-men had agglutinins and protective antibodies in the blood without previous jaundice.

In rabbits I found that agglutinins given by passive immunisation disappear quickly from the circulation. This was tested as a guide to the effect of therapeutic injections of serum in patients in the agglutination test.

I found that penicillin is lethal to *L. ictero-haemorrhagiae* in vitro and, from experiments partly done with Dr. J.C. Broom, concluded that penicillin must be started on the same day as infection to be therapeutically effective in guinea-pigs. Treatment by penicillin did not reduce the antibodies produced by infection.

Weil's disease does not, in my experience of sixty instances, cause a Wassermann reaction.

Prophylaxis against Weil's disease in sewer men in-

cludes reduction of rats, instruction in and opportunities for hygiene and warning of the infection to doctors who attend the men. The disease is scheduled under the Workmen's Compensation Act.

## PART II. LEPTOSPIROSIS CANICOLA.

*L. canicola* was identified as a new species by Klarenbeek and Schuffner in 1931. Throughout the world, it was found to be the cause of much infection in dogs, including dog typhus, or Stuttgart disease. It has not been found in any species of animal except dogs. Dogs may have severe, mild or unapparent infections and may pass the leptospiras in urine for weeks or months after any form of infection.

Bacteriological diagnosis is by culture of blood or urine, transfer of infection to hamsters or by agglutination tests of blood.

Human beings have been found infected in 145 instances known to me by published or unpublished records. Ninety-seven of these have been found in Holland and Denmark; ten have been diagnosed in the British Isles.

Dogs are always the source of human infections by people handling dogs, by dogs contaminating the floor, human food or water in which people bathe.

The clinical forms are an illness like influenza often with meningitis, conjunctival injection, or muscular pains.

Death has been recorded once in print, in Holland, and once in the British Isles (unpublished).



I have taken part in the serological diagnosis of five patients with leptospirosis canicola.

I found that *L. canicola* is sensitive to penicillin.

Search should be made in the British Isles for *L. ictero-haemorrhagiae* in animals, such as pigs, in whom it has been found elsewhere. Similarly, leptospiras other than *L. ictero-haemorrhagiae* should be sought in this country, in rodents in whom such species have been discovered abroad.